AQUATIC EFFECTS TECHNOLOGY EVALUATION (AETE) PROGRAM

Evaluation of Toxicity Reduction Evaluation (TRE) and Toxicity Identification Evaluation (TIE) Application to the Canadian Mining Industry

AETE Project 1.2.5

EVALUATION OF
TOXICITY REDUCTION EVALUATION (TRE) AND TOXICITY IDENTIFICATION EVALUATION (TIE) APPLICATION TO THE CANADIAN MINING INDUSTRY

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AQUATIC EFFECTS TECHNOLOGY EVALUATION PROGRAM

Notice to Readers

Evaluation of Toxicity Reduction Evaluation (TRE) and Toxicity Identification Evaluation (TIE) Application to the Canadian Mining Industry

The Aquatic Effects Technology Evaluation (AETE) program was established to review appropriate technologies for assessing the impacts of mine effluents on the aquatic environment. AETE is a cooperative program between the Canadian mining industry, several federal government departments and a number of provincial governments; it is coordinated by the Canada Centre for Mineral and Energy Technology (CANMET). The program was designed to be of direct benefit to the industry, and to government. Through technical and field evaluations, it identified cost-effective technologies to meet environmental monitoring requirements. The program included three main areas: acute and sublethal toxicity testing, biological monitoring in receiving waters, and water and sediment monitoring.

The technical evaluations were conducted to document certain tools selected by AETE members, and to provide the rationale for doing a field evaluation of the tools or provide specific guidance on field application of a method. In some cases, the technical evaluations included a go/no go recommendation that AETE takes into consideration before a field evaluation of a given method is conducted.

The technical evaluations are published although they do not necessarily reflect the views of the participants in the AETE Program. The technical evaluations should be considered as working documents rather than comprehensive literature reviews. The purpose of the technical evaluations focussed on specific monitoring tools. AETE committee members would like to stress that no one single tool can provide all the information required for a full understanding of environmental effects in the aquatic environment.

For more information on the monitoring techniques, the results from their field application and the final recommendations from the program, please consult the AETE Synthesis Report to be published in February 1999.

Any comments concerning the content of this report should be directed to:

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PROGRAMME D'ÉVALUATION DES TECHNIQUES DE MESURES D'IMPACTS EN MILIEU AQUATIQUE

Avis aux lecteurs

Examen des méthodes d'évaluation de réduction de la toxicité (ERT) et d'identification de la toxicité (EIT) pour l'industrie minière canadienne

Le Programme d'évaluation des techniques de mesure d'impacts en milieu aquatique (ÉTIMA) visait à évaluer les différentes méthodes de surveillance des effets des effluents miniers sur les écosystèmes aquatiques. Il est le fruit d'une collaboration entre l'industrie minière du Canada, plusieurs ministères fédéraux et un certain nombre de ministères provinciaux. Sa coordination relève du Centre canadien de la technologie des minéraux et de l'énergie (CANMET). Le programme était conçu pour bénéficier directement aux entreprises minières ainsi qu'aux gouvernements. Par des évaluations techniques et des études de terrain, il a permis d'évaluer et de déterminer, dans une perspective coût-efficacité, les techniques qui permettent de respecter les exigences en matière de surveillance de l'environnement. Le programme comportait les trois grands volets suivants : évaluation de la toxicité aiguë et sublétale, surveillance des effets biologiques des effluents miniers en eaux réceptrices, et surveillance de la qualité de l'eau et des sédiments.

Les évaluations techniques ont été menées dans le but de documenter certains outils de surveillance sélectionnés par les membres de l'ÉTIMA et de fournir une justification pour l'évaluation sur le terrain de ces outils ou de fournir des lignes directrices quant à leur application sur le terrain. Dans certains cas, les évaluations techniques pourraient inclure des recommandations relatives à la pertinence d'effectuer une évaluation de terrain que les membres de l'ÉTIMA prennent en considération.

Les évaluations techniques sont publiées bien qu'elles ne reflètent pas nécessairement toujours l'opinion des membres de l'ÉTIMA. Les évaluations techniques devraient être considérées comme des documents de travail plutôt que des revues de littérature complètes. Les évaluations techniques visent à documenter des outils particuliers de surveillance. Toutefois, les membres de l'ÉTIMA tiennent à souligner que tout outil devrait être utilisé conjointement avec d'autres pour permettre d'obtenir l'information requise pour la compréhension intégrale des impacts environnementaux en milieu aquatique.

Pour des renseignements sur l'ensemble des outils de surveillance, les résultats de leur application sur le terrain et les recommandations finales du programme, veuillez consulter le Rapport de synthèse ÉTIMA qui sera publié en février 1999.

Les personnes intéressées à faire des commentaires concernant le contenu de ce rapport sont invitées à communiquer avec M^{me} Geneviève Béchard à l'adresse suivante :

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EXECUTIVE SUMMARY

The Aquatic Effects Technology Evaluation (AETE) Program commissioned a study to evaluate and summarize the experience of the Canadian mining industry with Toxicity Identification/Reduction Evaluations (TI/REs). The objectives were: i) to complete a critical evaluation of the quality of TI/RE data, its benefits and limitations and, ii) to conduct a survey to evaluate the utility of the TI/RE strategies, including discussions on TIEs and effluent treatment, in determining and/or addressing aquatic impacts from mining operations.

A review of the scientific literature indicated little pertinent published information directly related to Canadian mining and TI/RE studies and therefore the application of TI/REs to Canadian mines could not be assessed on this basis. However, the limited number of articles reviewed in this document should not give the impression that there are few evaluations being conducted, since for various reasons the overwhelming majority of studies are never published in the scientific literature.

A complete assessment of the Canadian mining sector's experience with the TI/RE process was not possible since less than 50% of mines responded to the survey. Of 42 mines which responded, only 25 (57%) reported having experienced acute toxicity. Of those mines reporting toxic effluents, 7 (28%) indicated that a TRE had been conducted and 17 (76%) reported having conducted at least one Phase I TIE. Very few mines reported going beyond the Phase I toxicity characterization. Of the 25 mines that reported their effluents as being toxic, 9 (36%) reported that toxicity was consistent, compared with 16 (64%) that experienced transient toxicity. Ammonia was the most commonly identified contaminant of concern and effluent toxicity appeared to be highly pH dependent. Several mines reported difficulties with the identification of secondary causes of toxicity. Five mines reported making changes to their treatment system or process based on the results of the TI/RE. Two mines reported that toxicity was eliminated following the changes, one mine reported that toxicity was reduced and two mines reported no change in toxicity. The treatment system or process changes varied from product substitution to implementation of a full-scale effluent treatment facility.

Five TI/RE case studies (CS) were also selected for detailed review. The main objective was to provide examples of TI/REs conducted by Canadian mines and the rationale behind their relative success or lack of success in implementation in terms of toxicant identification, effluent treatment changes and toxicity reduction or elimination.

In CS #1, a copper/zinc mine, the primary toxicants were identified as copper and ammonia, but secondary toxicants (silver, aluminum and total dissolved solids (TDS)) were also suspected. The TIE lead to the identification of a strategy for reduction of ammonia toxicity to rainbow trout, the main concern of the client. The mine closed, but effluent continues to be discharged and is occasionally toxic.

In CS #2, a uranium mine, the primary toxicant was identified as an aliphatic alcohol (isodecanol). This lead to process modifications and modifications to the treatment system to solve the toxicity problem. The effluent is currently nonlethal to trout.

In CS #3, a copper/nickel mine, the primary toxicant was identified as ammonia, but secondary toxicants (metals) were suspected. The treatment system was augmented with pH control and toxicity was reduced. Mine personnel felt the results of the TIE were not worth the total cost of the studies since the conclusions were based mostly on speculation, rather than on statistically relevant results. Treatability studies provided more relevant and applicable information particularly related to establishing appropriate limits for pH. Historically, the effluent had been acutely lethal to trout or *Daphnia magna* in most toxicity tests. Most recently, the effluent has been consistently nonlethal to both species on all occasions of testing.

In CS #4, a gold mine, the general characteristics of the suspected toxicant(s) were identified (e.g., metals - most likely copper), but were not confirmed using the TIE process. Treatability investigations included bench scale evaluations, water reclamation and pilot plant studies. A full effluent treatment plant was installed based on the results of the treatability studies. However, the target level for copper used during these studies was at the *Daphnia magna* LC50. Effluent tested during the pilot plant trials indicated all treated samples were nonlethal to trout. However, partial daphnid mortalities (20%) were observed in those samples that exceeded the total copper target levels. During the most recent discharge period, the effluent was toxic to both rainbow trout and *Daphnia magna* (~ 80% mortality was observed). Ammonia, produced during the destruction of cyanide, is the suspected cause of trout mortality. Metals may be the cause of daphnid toxicity.

In CS #5, a cobalt/nickel and precious metals refinery, several possible causes of toxicity were suspected, but not conclusively identified. It was hypothesized that sodium levels were sufficient to account for at least 50% of the *Daphnia magna* mortality. Copper, potassium and carbonates were identified as potentially important factors in explaining daphnid mortality. Atypical ion balance was also a suspected cause of daphnid toxicity. Based on the limited available data, it was suspected that periodic peaks in sodium and/or copper concentrations contributed to the sporadic toxicity. The standard approach to toxicant identification was not possible since the U.S. EPA Phase I TIE treatments were ineffective at reducing or eliminating effluent toxicity. Subsequent toxicant identification efforts are in progress, but have required the development of innovative methodologies and techniques.

The following are the general conclusions and recommendations based on the survey responses and case studies regarding TI/REs as applied to the Canadian mining industry:

- TIEs do not "prove" the cause of toxicity, but rather use a weight of evidence approach.
- The TI/RE process is a systematic approach which incorporates the responses of organisms into the assessment of complex effluent mixtures to determine the identity of the substance(s) responsible for toxicity.
- Full transfer of information and communication between the mine and testing laboratory is

critical to the success of a TI/RE study.

- The overall success of a TI/RE is based on a number of factors including the experience of the laboratory personnel performing the tests and the variability in effluent quality.
- Laboratories should be equipped with all the basic and specialized laboratory equipment required to conduct the TI/RE and the personnel should be skilled and experienced in operating the equipment.
- Beyond Phase I, the TIE approach is not standardized and subsequent studies to identify the specific toxicants require experienced personnel.
- If identification and confirmation studies are to be successful, it is crucial that the tests are well planned and scientifically defendable. It is at these stages of the TI/RE study that the experience of the investigator is crucial.
- TI/REs are generally more likely to be successful when an effluent is consistently toxic, if the loss of toxicity is minimal over time and if the factors contributing to toxicity do not vary from one sample to the next.
- Conversely, the process can be rendered more difficult if toxicity is transient, if the samples quickly lose toxicity over time or if the factors contributing to toxicity are variable (i.e. different causative agents).
- Repeated testing is required to account for effluent variability and confirm that the cause of toxicity is the same under all conditions.
- Appropriate and relevant chemical analysis should be coordinated with toxicity testing on untreated and treated effluent samples.
- The lack of statistical comparisons may not be critical at certain stages of a TIE study, where gross changes in toxicity are the only consideration. However, large amounts of data can become unmanageable and difficult to interpret without statistical analysis. Multiple regressions are likely to yield better results for matrix dependent toxicants or in cases where multiple toxicants are suspected.
- The generation of a sufficient amount of data to provide strong evidence regarding the identification of the toxicant is critical if the mine is to consider investment in costly plant-scale remedial measures.
- Toxicity testing must be included in all bench scale and pilot plant studies.
- Modifications to the standard U.S. EPA Phase I TIE approach should be investigated and specific treatment methods or approaches developed for the Canadian mining industry.
- The use of rainbow trout in a Phase I study often requires greater effort and expense since

trout require large test volumes. However, surrogate test species (e.g., fathead minnows) may not be appropriate in a Canadian context. Modifications to the standard Environment Canada rainbow trout protocol should be developed and standardized for use with TIEs.

SOMMAIRE

Dans le cadre du Programme d'évaluation des techniques de mesure d'impacts en milieu aquatique (ETIMA), une étude visant à résumer l'expérience de l'industrie minière canadienne dans les domaines de l'évaluation des mesures d'identification des causes de toxicité (IT) et de réduction de la toxicité (RT) a été entreprise. Les objectifs visés étaient les suivants : i) réaliser une évaluation critique de la qualité des données amassées dans le cadre de programmes d'IT et de RT; ii) réaliser un sondage afin d'évaluer l'utilité des stratégies d'IT et de RT et des méthodes d'évaluation des mesures d'IT et de traitement des effluents pour la détermination et la résolution des problèmes causés aux écosystèmes aquatiques par l'exploitation minière.

Un examen de la documentation scientifique a mis en évidence le manque d'informations publiées pertinentes directement liées au secteur minier canadien et aux études consacrées aux stratégies d'IT et de RT. Par voie de conséquence, l'évaluation sur la base de ces informations de l'applicabilité des stratégies de d'IT et de RT au secteur minier canadien s'est révélée impossible. Toutefois, le nombre limité d'articles auxquels il est fait référence dans le présent document ne doit pas induire le lecteur à croire que la question suscite peu d'intérêt parmi la communauté scientifique. En réalité, pour diverses raisons, les résultats de la très grande majorité des études consacrées à la question ne sont jamais publiées dans les revues scientifiques.

Il a été impossible de réaliser une évaluation globale de l'expérience du secteur minier canadien dans les domaines de l'IT et de la RT puisque moins de 50 % des entreprises minières ont répondu au sondage. Parmi les 42 entreprises minières qui ont répondu à ce sondage, seulement 25 (57 %) ont affirmé avoir éprouvé ou éprouver des problèmes de toxicité aiguë. Parmi ces dernières, 7 (28 %) ont indiqué avoir procédé à une évaluation des mesures de réduction de la toxicité, et 17 (%) ont affirmé avoir achevé au moins la première étape du processus d'IT. Très peu d'entreprises minières ont poursuivi la caractérisation des causes de la toxicité au-delà de la première étape. Parmi les 25 entreprises minières qui ont affirmé rejeter des effluents toxiques, 9 (36 %) ont mentionné que les problèmes de toxicité étaient constants, tandis que 16 (64 %) ont indiqué que ces problèmes étaient intermittents. L'ammoniac a été le contaminant le plus fréquemment mentionné, et le degré de toxicité des effluents semblait étroitement lié au pH. Plusieurs entreprises minières ont éprouvé des difficultés à identifier les causes secondaires de toxicité. Cinq mines ont apporté des modifications à leur système ou procédé de traitement à la lumière des résultats de l'évaluation des mesures d'IT ou de RT. Ces modifications se sont révélées plus ou moins efficaces selon les mines : élimination complète des problèmes de toxicité (2 mines), réduction des causes de toxicité (1 mine) et aucun changement (2 mines). Les modifications apportées au système ou procédé de traitement ont également varié d'une mine à l'autre, allant d'une simple substitution de produit à la mise en place d'une installation de traitement des effluents à grande échelle.

Cinq études de cas consacrées à l'évaluation des mesures d'IT ou de RT ont également fait l'objet d'un examen approfondi. L'objectif visé était de fournir des exemples de telles études menées par des entreprises minières canadiennes et d'expliquer pourquoi ces études ont permis ou non d'identifier les causes de toxicité, d'apporter des modifications au système ou au processus de traitement des effluents et de réduire ou d'éliminer les problèmes de toxicité.

Étude ce cas n° 1, mine de cuivre et de zinc : le cuivre et l'ammoniac étaient les principales substances toxiques mises en cause, mais la présence de substances toxiques secondaires (argent, aluminium et TSD) était soupçonnée. L'évaluation des mesures d'IT a conduit à l'élaboration d'une stratégie visant à réduire la toxicité de l'ammoniac pour la truite arc-en-ciel, principale préoccupation du client. La mine a mis un terme à ses activités, mais les rejets se poursuivent et l'effluent est occasionnellement toxique.

Étude ce cas n° 2, mine d'uranium : un alcool aliphatique (isodécanol) était la principale substance toxique identifiée. La mine a apporté des modifications à ses procédés et système de traitement afin de résoudre ce problème de toxicité. L'effluent n'est actuellement plus toxique pour la truite.

Étude ce cas n° 3, mine de cuivre et de nickel : l'ammoniac était la principale substance toxique mise en cause, mais la présence de substances toxiques secondaires (métaux) était également soupçonnée. L'entreprise a modifié son système de traitement afin de le rendre plus efficace et introduit un mécanisme de régulation du pH, ce qui a permis de réduire les problèmes de toxicité. Le personnel de la mine estimait que les résultats de l'évaluation des mesures d'IT ne justifiaient pas le coût total de l'étude puisque les conclusions étaient essentiellement fondées sur des hypothèses plutôt que sur des résultats concrets fiables statistiquement. Les études de traitabilité ont permis d'obtenir des renseignements plus concluants se prêtant mieux à une application pratique, notamment en ce qui a trait à l'établissement de limites appropriées pour le pH. La majorité des épreuves de toxicité réalisées antérieurement avec des truites et des daphnies (*Daphnia magna*) avaient démontré que l'effluent posait des problèmes de toxicité aiguë. Tous les essais effectués depuis ont révélé que l'effluent n'est plus toxique pour ces deux espèces.

Étude ce cas n° 4, mine d'or : l'évaluation des mesures d'IT a permis de cerner les caractéristiques générales des substances toxiques présumées (p. ex., métaux, selon toute vraisemblance le cuivre) sans toutefois confirmer leur identité précise. Des études de traitabilité incluant des évaluations en laboratoire, des essais d'épuration d'eau et des essais d'installations pilotes ont été réalisées. Ces études ont débouché sur la mise en place d'une installation fonctionnelle de traitement des effluents. Toutefois, la concentration de cuivre visée dans le cadre de ces essais était la CL_{50} pour le *Daphnia magna*. L'ensemble des échantillons traités analysés durant les essais de l'installation pilote se sont révélés non létaux pour la truite. Toutefois, 20 % des daphnies exposées aux échantillons présentant des teneurs en cuivre total excédant la concentration visée sont mortes. Les dernières analyses d'échantillons d'eau provenant de l'effluent de la mine ont révélé que l'effluent est encore toxique (taux de mortalité observé de ~ 80 %) pour la truite arc-en-ciel et le *Daphnia magna*. L'ammoniac généré par la destruction des cyanures est soupçonné d'être à l'origine de la mortalité observée chez la truite arc-en-ciel, tandis que les métaux sont mis en cause dans la mortalité observée chez la daphnie.

Étude de cas n° 5, affinerie de cobalt/nickel et de métaux précieux : on soupçonne plusieurs substances d'être toxiques mais elles n'ont pas été identifiées de façon définitive. On avance l'hypothèse que les concentrations de sodium étaient suffisantes pour occasionner un taux de mortalité d'au moins 50 % de *Daphnia magna*. Le cuivre, le potassium et les carbonates ont été identifiés comme étant des éléments potentiellement importants qui expliqueraient le taux de mortalité des daphnies. Un bilan d'ions atypiques est également susceptible d'avoir été létal pour les daphnies. En se basant sur les données disponibles limitées, on soupçonne que les pics périodiques des concentrations de sodium et/ou de cuivre ont contribué à la toxicité sporadique. Il a été impossible d'utiliser l'approche normalisée pour identifier les substances toxiques puisque les traitements en vue d'identifier les causes de toxicité (IT) de la Phase I de l'EPA, É.-U. n'ont pas réussi à réduire ou à éliminer la toxicité de l'effluent. Les efforts ultérieurs qui ont été faits pour identifier la substance toxique se poursuivent mais ont exigé l'élaboration de méthodologies et de techniques novatrices.

Les réponses au sondage effectué auprès des entreprises minières et les résultats de l'examen des études de cas consacrées aux mesures d'IT et de RT prises par l'industrie minière canadienne ont inspiré les conclusions et recommandations suivantes :

- L'évaluation des mesures d'IT ne permet pas de « confirmer » la cause de toxicité, mais elle utilise une approche fondée sur la valeur des preuves.

- Le processus d'IT/RT est une approche systématique qui incorpore les réactions des organismes dans l'analyse de mélanges d'effluents complexes visant à identifier les substances toxiques en cause.

- La qualité du transfert d'informations et de la communication entre la mine et le laboratoire joue un rôle critique dans le succès de l'évaluation des mesures d'IT/RT.

- Le succès global d'une évaluation des mesures d'IT/RT dépend d'un certain nombre de facteurs, dont l'expérience du personnel du laboratoire chargé d'effectuer les essais et la variabilité de la qualité de l'effluent.

- Les laboratoires chargés d'effectuer les essais doivent être pourvus de l'équipement de base et du matériel spécialisé voulus pour mener à bien une évaluation des mesures d'IT/RT, et leur personnel doit être compétent et savoir comment faire fonctionner ces équipements.

- Au-delà de l'étape I, le processus d'évaluation des mesures d'IT n'est pas normalisé, et le succès des études subséquentes visant à identifier des substances toxiques particulières repose sur l'expérience du personnel.

- Pour que le processus d'identification et les études de confirmation se révèlent un succès, il est essentiel que les essais soient bien planifiés et justifiés scientifiquement. L'expérience du chercheur joue un rôle crucial à ces étapes du processus IT/RT.

- L'évaluation des mesures d'IT et de RT est habituellement plus efficace si les problèmes de toxicité posés par l'effluent se manifestent de façon constante, si la diminution dans le temps de la toxicité est minimale et si les facteurs responsables de la toxicité ne varient pas d'un échantillon à l'autre.

- À l'inverse, le processus peut perdre de son efficacité si les problèmes de toxicité surviennent de façon intermittente, si la toxicité des échantillons s'amenuise rapidement ou si les facteurs à l'origine des problèmes de toxicité varient (p. ex. agents causals différents).

- Pour prendre en compte la variabilité de la composition de l'effluent et confirmer que la cause des problèmes de toxicité demeure la même quelles que soient les conditions, il est essentiel de procéder à des essais répétés.

- Il importe d'effectuer des analyses chimiques appropriées de concert avec les épreuves de toxicité pour tous les échantillons d'effluent traités et non traités.

- L'absence de comparaison statistique peut ne pas être critique à certaines étapes du processus d'IT/RT dans les cas où les changements grossiers de toxicité représentent la seule considération. Toutefois, le traitement et l'interprétation d'un grand nombre de données peut être difficile en l'absence d'analyse statistique. Les analyses par régressions multiples sont plus susceptibles de fournir de meilleurs résultats dans le cas où la toxicité des substances considérées dépend de la matrice ou dans le cas où la présence de plusieurs substances toxiques est soupçonnée.

- Il est essentiel que la mine obtienne suffisamment de données pour confirmer l'identification de la (des) substance(s) toxique(s) avant d'investir des sommes considérables dans la mise en place de mesures correctrices coûteuses intéressant l'ensemble de l'usine.

- Tous les essais en laboratoire et les essais d'installations pilotes doivent comporter des épreuves de toxicité.

- Il convient d'apporter des modifications à l'étape I du processus d'IT proposé par l'EPA des États-Unis et d'élaborer de nouvelles méthodes ou approches de traitement à l'intention de l'industrie minière canadienne.

- L'utilisation de truites arc-en-ciel dans le cadre de l'étape I d'une étude est fastidieuse et onéreuse, car il faut prévoir des volumes d'eau plus importants pour les essais. Toutefois, le remplacement de la truite arc-en-ciel par une autre espèce (p. ex. tête-de-boule) peut ne pas convenir aux conditions particulières qui prévalent au Canada. Il convient de proposer et de normaliser des modifications au protocole d'essai normalisé d'Environnement Canada s'appliquant à la truite-arc-en-ciel en vue d'en favoriser l'utilisation dans le cadre du processus d'IT.

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1. INTRODUCTION

1.1 Background

The AETE program was established to review appropriate technologies for assessing the impacts of mine effluents on the aquatic environment. AETE is a cooperative program coordinated by the Canada Centre for Mineral and Energy Technology (CANMET). The program was designed to evaluate and identify cost-effective technologies to meet environmental monitoring requirements. The program included three main areas: acute and sublethal toxicity testing, biological monitoring in receiving waters and water and sediment monitoring. An important part of each program is to identify effective methods that provide the required information at the least cost.

The issues of Toxicity Identification and Reduction Evaluations (TI/REs) were discussed by AETE members. TIEs are a major component of TREs in which toxicity tests are combined with chemical analyses to identify and confirm causative toxicants. TIEs are usually commissioned by industry when an effluent is acutely toxic or exerts a sublethal response. Toxicity Treatability Evaluation (TTEs) are another major component of TREs in which the specific causative toxicant(s) may not be known, but the characteristics of the toxicant are known and lead the investigator to evaluate specific toxicity treatability techniques to eliminate toxicity. A successful TRE investigation will lead to toxicity source identification (i.e., remediation through process change or by chemical substitution) or effluent treatment. Standard biological test methods, which measure various responses such as lethality, growth and reproduction for marine and freshwater species, are used in both acute and sublethal TIEs.

1.2 Objectives

The overall objective of this study was to evaluate and summarize the experience of Canadian mining industries with effluent TREs and TIEs in attempting to resolve acute lethality and sublethality problems. The specific objectives were as follows:

i) to complete an overall critical evaluation of the quality of TI/RE data, its benefits and limitations and,

ii) to conduct a survey to evaluate the utility of the TI/RE strategies, including discussions on TIEs and effluent treatment, in determining and/or addressing aquatic impacts from mining operations.

1.3 Outline of the TI/RE Process

The toxicity based TI/RE process combines on-site source evaluations with toxicity testing and chemical analysis to provide a comprehensive approach to identifying the source and cause of effluent toxicity. The general objectives of the TI/RE process are to i) evaluate the potential sources of toxicity, ii) characterize the toxicity observed in the sample, iii) provide a preliminary identification of the possible sources of this toxicity by evaluating changes that occurred in the toxicity following a variety of chemical and physical manipulations and treatments, and iv) ultimately provide measures for reduction and elimination of the toxicants (U.S. EPA 1989).

A successful TRE will involve the coordination of a multi-disciplinary team including toxicologists, chemists, engineers and very importantly, mine personnel. The steps involved in successfully meeting the TRE objectives are outlined in Figure 1-1.

The TIE portion of the TRE program is divided into three phases. Phase I involves characterization of the toxicants through a variety of effluent treatments (U.S. EPA 1991a). Phase II involves identification of the suspected toxicant(s) (U.S. EPA 1993a). Confirmation of the suspected toxicants occurs in Phase III (U.S. EPA 1993b). Phase I, II and III usually occur sequentially, but are often done simultaneously when patterns of toxicity begin to emerge using Phase I. In some cases, however, following the Phase I characterization, a decision may be made to proceed directly to an effluent toxicity treatability evaluation.

Figure 1-1. Outline of the TI/RE process

During Phase I, toxicity of the untreated effluent (baseline test) is compared to the treated sample following the effluent manipulations. The relative degree to which the manipulations result in an improvement in effluent effects provide an indication of the types of contaminants that may be involved (e.g., volatiles, heavy metals, organics). Toxicity of the various manipulated effluent samples is assessed by determining the acute lethal or sublethal response of the test organism to the treated or untreated effluent.

Chemical analysis of the effluent can be conducted at various stages during a TIE study. For example, preliminary analysis of the effluent collected during toxicity testing can be very useful, particularly if mine personnel have reason to suspect a particular toxicant (e.g., ammonia, metals). However, detailed chemical analysis is generally most effective once the characteristics of the toxicant have been identified.

PHASE I

The Phase I TIE methods were originally developed for use with acute lethality tests using fathead minnows or *Ceriodaphnia dubia*, but have recently been adapted for sublethal and sediment testing (U.S. EPA 1991b,c). A list of the "standard" U.S. EPA TIE Phase I effluent characterizations are provided in Table 1-1 and a brief description of each treatment is provided below.

Table 1-1. List of U.S. EPA TIE Phase I effluent characterization treatments

TREATMENTS
Graduated pH
Filtration at pH_i (initial pH of the effluent), 3 and 11
Aeration at pH_i, 3 and 11
C18 Solid Phase Extraction pH_i, 3 and 9
Treatment with EDTA
Treatment with Sodium Thiosulfate

A significant portion of the toxicity observed in industrial effluents can often be influenced by pH. Changes in pH can have profound effects on a number of chemical and physical properties of toxicants including solubility, polarity, speciation and stability, properties which are accounted for in the Phase I characterization treatments. pH can also change the ratio of ionized to un-ionized forms of toxicants with a resultant change in toxicity. For example, the un-ionized form of ammonia is considerably more toxic than the ionized form. Concentrations of un-ionized ammonia are dependent on pH, and to a lesser extent upon temperature. As pH increases, the proportion of un-ionized ammonia increases (Thurston *et al.* 1981). Changes in pH can also affect the equilibrium between metal ion complexes which can in turn affect solubility, bioavailability and toxicity. pH adjustment is used throughout the Phase I to provide more information on the nature of the toxicant(s). The pH adjustment tests also act as blanks for subsequent Phase I pH adjustment tests performed in combination with other treatments (U.S. EPA 1991a).

i) pH Adjustment / Graduated pH

This test evaluates the effect of pH on the toxicity of a variety of contaminants, particularly ammonia and often metals. Sub-samples of the effluent are adjusted to a low, medium and high pH within the tolerance range of the test organism (e.g., usually 6-9) and then tested for toxicity without pH re-adjustment.

ii) pH Adjustment/Filtration Treatment

The pH adjustment and filtration tests evaluate the effect of pH change and filtration on the toxicity of the effluent. The pH of sub-samples of effluent is adjusted to 3 and 11 using solutions of NaOH or HCl as required. Glass fibre 1.0 μm filters are prewashed with deionized water and the pH unadjusted and adjusted effluent samples are filtered. The pH of each effluent sample is then re-adjusted to the initial pH of the sub-sample (pH_i) and the toxicity tested.

iii) pH Adjustment/Aeration

The pH adjustment and aeration tests evaluate the effect of pH change and aeration on the toxicity of a sample that may be due to compounds that can become oxidizable or volatile after pH adjustment. In this test, sub-samples of the pH unadjusted and pH adjusted effluent (pH 3 and 11) are aerated for one hour. Following aeration, the pH of each effluent sub-sample is then re-adjusted to pH_i and the toxicity tested.

iv) pH Adjustment / C18 Adsorption

This test evaluates the extent to which toxicity may be due to relatively non-polar organics and certain metals. A sub-sample of effluent at pH_i is passed through a prepared C18 cartridge and non-polar contaminants are adsorbed onto the C18 material. Sub-samples are adjusted to a pH of 3 and 9 and are also passed through prepared C18 cartridges. This pH adjustment will cause any organic acids and bases that are present to become less polar by shifting the chemical equilibrium to the un-ionized species. Following treatment, the sub-samples are re-adjusted to pH_i and tested for toxicity.

v) EDTA Chelation Treatment

Ethylenediaminetetraacetate (EDTA) has the ability to combine with a variety of metals and produce relatively non-toxic complexes. This ability is due to the chelation properties of EDTA and is used to remove effluent toxicity potentially associated with metals such as copper. The amount of EDTA required is dependent on the concentrations of metals present and the toxicity of EDTA to the test species. Recent studies have indicated that the EDTA binding capacity is not affected by the presence of calcium or magnesium. To determine the effects of EDTA addition, toxicity screening tests are conducted on 100% effluent containing a range of EDTA concentrations. Following the addition of EDTA, the sub-samples are re-adjusted to pH_i and tested for toxicity.

vi) Sodium Thiosulfate Treatment

Oxidative substances (e.g. chlorine, iodine, bromine) can be made less toxic or non toxic by the addition of sodium thiosulfate. Recent studies have also suggested that toxicity from certain metals (e.g. copper, silver and selenite) can be effectively reduced with the addition of sodium thiosulfate (Hockett and Mount 1996). Varying amounts of sodium thiosulfate are added to sub-samples of the effluent. Following the addition of sodium thiosulfate, the sub-samples will be re-adjusted to pH_i and tested for toxicity.

PHASE II

In Phase II of a TIE, further effluent treatments are conducted to identify the specific substance(s) responsible for toxicity. Analytical methods are used to obtain a quantitative measurement of the suspected toxicants. The additional treatments and analytical methods chosen are directly related to those treatments observed to effectively eliminate or reduce toxicity during Phase I. An important component of Phase II is the "tracking" of toxicity on all untreated and treated effluent samples. While there are many possible approaches to Phase II, examples of approaches suggested by the U.S. EPA are briefly described in the following section.

Ammonia may be suspected if toxicity decreased or was eliminated at low pH during the graduated pH test. Subsequent Phase II testing can involve the use of zeolite to selectively remove ammonia from the effluent. Zeolites are crystalline aluminosilicates which exhibit high selectivity for ammonia, but can remove some heavy metals (Sherman 1978). Samples of effluent are analyzed for ammonia before and after treatment with zeolite using appropriate analytical techniques (e.g., specific ion or colourmetric electrodes). Similarly, if EDTA was effective at removing toxicity, then the appropriate analytical techniques would be applied to analyze for metals (e.g., inductively coupled plasma/mass spectrometry (ICP/MS), atomic absorption (AA)).

If aeration effectively reduced or eliminated toxicity, further testing would be conducted to determine if the substance was removed through oxidation, volatization or sublation. The use of nitrogen for sparging of the sample will result in the displacement of gases that are volatile and eliminate oxidation as a removal process. If the nitrogen treatment removed toxicity then a volatile substance(s) may be responsible. If toxicity is reduced by sparging with oxygen and not with nitrogen, oxidation is the likely cause. If toxicity is recovered when the sides of the aeration cylinder are rinsed using deionized water, methanol, sodium hydroxide or hydrochloric acid, then toxicity may be related to sublatable substances. Sublatable substances can be defined as surface active agents that can be "lifted or carried" out of solution (U.S. EPA 1991a).

In cases where C18 was effective at reducing or eliminating toxicity, non-polar organics may be the cause of toxicity. The material bound to the C18 sorbent can be isolated by eluting (rinsing) the column with varying concentrations of methanol, thereby concentrating the substance(s) responsible for toxicity. A portion of the methanol concentrates would be tested for toxicity, while a second portion is sent for chemical analysis (e.g., gas chromatography/mass spectrometry (GC/MS), high pressure liquid chromatography (HPLC), liquid chromatography/mass spectrometry (LC/MS)).

PHASE III

Phase III of a TIE study involves a series of steps to assist in confirming that the suspected toxicant(s) are correctly identified and that all toxicity is accounted for. As with Phase II, there are many possible approaches to confirming the substance(s) responsible for effluent toxicity. The guidance provided by the U.S. EPA includes, but is not limited to, the use of: i) mass balance, ii) correlation, iii) symptom approach, iv) comparison of species sensitivity and v) spiking effluent with suspected toxicant(s). Examples of approaches suggested by the U.S. EPA are briefly described in the following section.

The mass balance approach is used when the toxicant(s) can be effectively removed from the effluent and subsequently recovered (e.g., C18 column treatment). Toxic and nontoxic fractions, expressed as toxic units (TUs), are summed and compared to the toxicity of the whole effluent to determine if all toxicity is account for. For effluent samples, TUs are obtained by dividing 100%

by the effect concentration (e.g., LC50). For the suspected toxicant, TUs are obtained by dividing the actual concentration of the suspected toxicant in solution by the threshold effect concentration for that toxicant. The purpose of the correlation approach is to determine if there is a consistent relationship between the concentration of the suspected toxicant(s) and effluent toxicity. Whole effluent toxicity is compared to the amount of suspect chemical(s) concentration.

The symptom approach includes the use of organism behaviour and time to death in comparing the responses of organisms to the whole effluent and then to the suspected toxicant(s). Differences in species sensitivity can be used to provide further evidence as to the cause of effluent toxicity (e.g., trout are generally more sensitive to ammonia than daphnids). Similarly, when two or more species exhibit different sensitivities to the suspected toxicant during single chemical testing, and the same pattern is observed in the whole effluent, this provides supporting evidence that the chemical tested is the cause of effluent toxicity (Ankley and Schubauer-Berigan, 1995). In spiking tests, the concentration of the suspected toxicant(s) is increased in the sample to determine if toxicity is increased in proportion to the increase in concentration. Alternatively, the suspected toxicant can be added to a nontoxic sample or to a sample of effluent where the suspected toxicant(s) has been removed. As with the symptom and species sensitivity approaches, these tests are not conclusive, but provide supporting evidence that the suspected toxicant is in fact the cause of effluent toxicity.

In the case where a toxicity treatability evaluation is the appropriate course of action, the investigator will usually evaluate the removal performance of different effluent treatment technologies at the bench-scale (e.g., in the laboratory). Toxicity removal technologies might include activated carbon, biological treatment, reverse osmosis, alum addition, advance oxidation processes etc. Once toxicity removal has been demonstrated at the bench scale level, a decision can be made to pilot the technique on a larger scale at the plant site. At this point, the toxicity removal technique can be assessed for compatibility with the existing system.

2. STUDY APPROACH AND METHODOLOGY

To meet the specific objectives of this evaluation, work requirements were divided into 5 tasks:

- Review of published and "grey" literature
- Survey and case studies
- Summary and description of TRE treatment options
- Summary and description of novel TIE manipulations
- Preparation of bibliography and glossary

An initial meeting with the AETE Secretariat, Toxicity Sub-Group and ESG International project director and manager was held to finalize the study approach. A description of the approved approach used for each task is provided in the following sections.

2.1 Review of Published and "Grey" Literature

The main objective of the literature review was to objectively and critically review published and "grey" literature. A restricted survey was undertaken involving referral to readily accessible databases. This survey involved a computerised search of the engineering and scientific databases, Zoological Abstracts, Engineering Index and Medical Index from the years 1989 to 1996. Current Contents was used to search for more recent publications (1997). The restricted nature of the survey, while it covers a large proportion of the literature published in scientific journals, includes few conference proceedings where TIE studies would be expected to form part of the agenda (e.g., Society of Environmental Toxicology and Chemistry meetings, Canadian Aquatic Toxicity Workshops).

Most of the grey literature is not widely distributed or readily available. Since these publications are rarely cited in computerised databases, there is little representation of the grey literature in the limited survey undertaken. Publications that are not normally available on a computerised abstract service, such as professional and industry magazines were in general not covered by this search.

The diversity of sources selected for this review negated the effectiveness of the suggested checklist approach. This approach is a useful tool if the many studies are selected which have major points in common (one toxicant, one industrial sector), yet vary in details (type of manipulations/differing experimental conditions). The variation in TIE studies limited the usefulness of this approach, since often the common points in the studies were in the details (e.g., selection of organisms; sample manipulations), but there were major differences in the fundamentals (type of waste, industrial sector).

2.2 Survey and Case Studies

The main objective of this task was to evaluate the overall success or failure of TREs and TIEs conducted by the Canadian mining industry (Appendices A and B). This task was divided into two sub-tasks; i) a survey questionnaire to be sent to mine operators and ii) detailed case studies. Although many TI/RE studies conducted to date have been completed in the United States, these studies commonly use fathead minnows and *Ceriodaphnia dubia* as the test species. In comparison, Canadian mines are more familiar with the use of the rainbow trout and *Daphnia magna* for biological assessment in regulatory monitoring and in TIE treatment methodologies which have been adapted from the U.S. EPA methods. It was therefore decided that this portion of the study would focus on Canadian TI/REs and limit U.S. contacts to a small number of American consultants (Russ Hockett, ENSR) and researchers (Dave Mount, U.S. EPA Duluth Laboratory) who would be asked about their experience with TI/REs as applied to the mining industry.

2.2.1 Mine Operator Survey Approach

The purpose of the survey was to collect and summarize information on TI/REs commissioned by the Canadian mining industry. The survey included questions that evaluated: i) the outcome of

TI/RE results (including those conducted on both acutely lethal and sublethal effluents); ii) the frequency of TI/RE use and costs incurred; and iii) the pertinent information and knowledge gained.

A concise questionnaire was developed with the input and approval of the AETE Toxicity Sub-Group. Questions were kept simple (e.g., Yes/No response) to encourage compliance and a rapid reply. A covering letter (from Natural Resources Canada and the Mining Association of Canada (MAC)) describing the objectives of the survey and the TI/RE process, accompanied each questionnaire. The name and telephone number of an AETE and mining industry representative was also provided. A copy of the covering letter and questionnaire are provided in Appendix B. All questionnaires were faxed directly to appropriate mine personnel whose names were provided to ESG by MAC, CANMET and Environment Canada. Mine personnel were allowed a four week period in which to complete the survey. Following the final data for survey submission, follow-up telephone calls were also made to selected mines in an attempt to increase the number of surveys completed and returned.

To ensure complete confidentiality, survey responses were identified only by mine type. Responses to each question in the survey were compiled and summarized to assess the application and overall degree of TI/RE success and failure. A selected number of mines which completed the survey were also contacted directly to obtained further detailed information on what they felt were the benefits and limitations of the TI/RE process

2.2.2 TI/RE Case Studies

The main objective of this task was to provide examples of TI/RE case studies and the rationale behind their relative success or lack of success in implementation in terms of effluent treatment changes, toxicity reduction and cost effectiveness. A total of five case studies were selected and reviewed. An attempt was made to select the case studies according to the following scenarios:

i) Specific toxicant identified and toxicity of the final effluent was eliminated.

ii) Specific toxicant identified, but toxicity of the final effluent was not eliminated.

iii) General characteristics of toxicant identified (e.g., organic, metal) and toxicity of the final effluent was eliminated through modification to process or external treatment system.

iv) General characteristics of toxicant identified (e.g., organic, metal), but toxicity of the final effluent was not eliminated through modification to process or external treatment system.

The selection of case studies also took into consideration the type of mine (e.g., gold, copper). Due to time constraints, the first case study was selected from suggestions provided to ESG by the Toxicity Sub-Group. The remaining four case studies were selected from the survey responses.

Information for each case study was obtained from a thorough review of all TI/RE reports, telephone interviews with mine personnel, telephone discussions with laboratory personnel conducting effluent manipulations, chemical analysis and testing, and where possible, discussions with the engineers who implemented the process or "end-of-pipe" treatment modifications.

The information obtained was objectively and critically evaluated using a checklist approach and a pre-approved "Evaluation Criteria List" (Appendix A). The checklist approach was considered a useful tool for evaluating the strengths and weaknesses of TREs and TIEs as applied to mining effluents as it will assist in identifying the most successful TIE treatment options. An added advantage was that the same criteria could be used by individual mines to assess the progress, success and/or failure of site-specific TIEs.

2.3 Summary and Description of TRE Treatment Options

The main objective of this task was to provide a brief summary and description of successful TRE treatment options encountered during the literature review, survey and case studies. Information was also obtained from direct conversations with mine personnel. The treatment options were selected based on those observed to successfully reduce or eliminate toxicity.

The ultimate goal of the TRE is to reduce toxicity in the final effluent. Initially, direct solutions to accomplish this are examined including housekeeping, chemical substitution and treatment plant optimization. Once these steps are completed, if the effluent is still toxic then other approaches are required. These may include source reduction technologies, assuming a specific source (e.g., process stream) can be identified, and improvement of waste treatment operations (e.g., biological treatment, additional settling time, pH adjustments). Cost, performance, ease of implementation, flexibility of the modification and expected life of the modification are included in the selection of the most appropriate toxicity reduction options (U.S. EPA 1989).

2.4 Summary and Description of Novel TIE Manipulations

The main objective of this task was to provide a summary and description of novel TIE techniques for use with mining effluents. Information gathered during the literature review, survey and case studies as well as ESG's own expertise and experience in conducting TI/REs, was used to prepare the summary.

2.5 Preparation of Bibliography and Glossary

A computer database, Procite®, was used to list references dealing with all aspects of TIE/TREs and the mining industry. A database of references was established at the start of study and was to be expanded during the project. Records of publications in the database included lists of keywords that were used as the basis for the glossary of terms.

3. RESULTS

3.1 Literature Review

The TIE literature is not extensive and there are very few published studies which deal with TIEs conducted for the mining industry. The issue of a lack of readily available published TI/RE information was confirmed during recent discussions with one of the original researchers responsible for the development of the TI/RE protocol (pers. comm. Teresa Norberg-King, U.S. EPA Duluth Laboratory). A selection of articles was made based on knowledge of the characteristics of mining effluents and the importance of the suspected or identified toxicant in relation to mining effluents. For example, ammonia is a common toxicity problem faced by many industrial sectors, including some mines. Some articles dealing with TIEs and ammonia toxicity are discussed, despite the fact that the industrial setting is different (pulp and paper for example). A short section on unidentified toxicants is included, as well as examples of TRE/treatability studies taken from a number of diverse industries.

i) Historical Background

TIEs are a relatively recent approach to effluent toxicity problems and most of the studies in this review are dated after 1993. The original U.S. EPA documents describing Phase I manipulations appeared in 1989, with further editions appearing in 1991 and 1993. Phase I TIE methods were originally developed for use with acute lethality tests using fathead minnows or *Ceriodaphnia dubia.* The TIE approaches have been suggested for chronic and sediment (pore water) testing (U.S. EPA 1991b, 1991c; Vansprang and Janssen 1997).

In the United States, the Phase I TIE is considered as the next phase of regulation for effluents that do not pass initial toxicity tests. Not all countries follow the U.S. EPA TIE approach, though it has influenced approaches used in other jurisdictions. For example, in Sweden, effluents can be classified on the basis of environmental effects using acute toxicity, biodegradation, and bioaccumulation criteria (Brorson *et al.* 1994). Analysis of the effluent includes chemical characterisation before and after treatment, comparisons with the toxicological literature to evaluate the impact of known substances and a limited set of tests, including the Microtox®, to estimate the effects of unknown parameters in the effluent (Brorson *et al.* 1994). In Britain, the use of toxicity tests for evaluation of the effects of an effluent on the environment, or for monitoring the performance of an effluent treatment plant, are becoming more important to complement the analytical techniques which are normally used (Lankford and Smith 1994).

This survey uncovered few TIE studies using Canadian examples and most of these dealt with pulp and paper effluents. Riebel *et al.*(1996) reviewed effluent toxicity prevention and reduction programs for Canadian pulp and paper mills. The authors describe the TIE / TRE procedures published by the U.S. EPA and recommend more cost-effective TIE methods adapted to pulp and paper mill effluents.

This review is organised primarily according to the type of toxicant suspected or identified in the particular study. These toxicants have been chosen because of their application to some mining effluents. For example, a great deal of recent literature deals with the problem of produced waters associated with the petroleum industry. These are high ionic strength effluents and in this characteristic they are similar to some mining effluents. Other sections deal with examples of studies involving TIEs and TREs of different industrial sectors. While the details of the studies are specific to one industry or even a single plant, the principle can be applied to other sectors.

ii) Elevated Total Dissolved Solids (Ionic Strength) / Produced Waters

As mentioned above, produced waters are high ionic strength waters associated with petroleum discharges. The toxicity of these discharges, which typically contain 10,000 mg/L as total dissolved solids (TDS), is mostly related to the constituents and not just to the elevated ionic strength (Mount *et al.* 1997). Total dissolved solids are difficult to address using existing TIE methodologies. McCulloch *et al.* (1993) investigated the toxicity of TDS and confirmed that elevated TDS alone can be a cause of toxicity. For freshwater animals, TDS toxicity is a result of osmotic stress and its impact on the osmoregulatory capability of the organism. The authors point out that TDS may be rapidly diluted below toxic levels in many situations and thus may have less impact on a receiving water than regulatory tests predict.

Other studies have applied regression techniques to identify the principle components causing toxicity during exposures in these types of effluents (Mount and Gulley 1992, Mount *et al.* 1997). The authors employed a logistic multiple regression model to relate mortality to the concentrations of total dissolved solids (TDS) present as ions (Na Cl, Ca Mg, etc.). To be successful, these techniques require an extensive database of toxicological and chemical results and their application is not part of the standard TIE approach. However, the ion concentrations in these models were entered as mg/L and the authors state that in many cases the multiple regression model overestimated the toxicity of the salt solutions to fish (Mount *et al.* 1997). This method of expressing concentrations is not correct if the contributions to toxicity of different ions or compounds in a mixture are to be properly compared and it is likely that the poor fit of the model can be partly explained by the use of incorrect concentration units.

The importance of ionic strength as a toxic stress was also reported by Douglas *et al.* (1996) who conducted a TIE on effluent from a petrochemical plant which discharges into an estuary. Ionic imbalance was also suspected as a source of toxicity, since the effluent had been consistently toxic to mysids (*Mysidopsis bahia*) but not to sheepshead minnows (*Cyprinodon variegatus*). Phase I effluent toxicity characterisation tests revealed that treatment of the effluent with a cation exchange resin was only partially effective at reducing toxicity. Phase II characterisation tests revealed that the degree of toxicity varied with the concentration of four cations. Toxicity was increased when concentrations of Ca and Sn were elevated. In contrast, toxicity decreased with elevated levels of Mg and K. A reduction in levels of Ca was shown to improve survival, but some toxicity still remained. Toxicity tests with individual constituents revealed that the level of Sn in the effluent was much less than the toxic threshold for the element. If the effluent concentrations of Ca, Mg, K and Br were adjusted to resemble natural seawater concentrations, all of the exposed organisms survived. Experiments with simulated effluents confirmed that ion imbalance was the only cause of toxicity in the effluent.

However, Sauer *et al.* (1997) studied several produced water samples of various salinities and concluded that other components in these effluents contributed to toxicity. Their TIE investigations suggested that no one component was responsible for toxicity in all samples and implicated salinity, acidic and basic organic compounds, particulates (removed by filtration at pH 11), ammonia, hydrocarbons, hydrogen sulfide, and volatile compounds. Some unidentified toxicants were removed simply by manipulating the pH (Sauer *et al.* 1997).

Ho *et al.* (1995) studied the effects of salinity adjustment and effluent storage on the toxicity of effluents during marine TIEs. The effects of storage with and without brine addition were determined using a municipal and an industrial effluent. There was less change in the toxicity of the effluents to Atlantic silverside (*Menidia beryllina*) and mysid shrimp (*Mysidopsis bahia*) if brine was added prior to storage. The authors also investigated the effect of brine addition on various TIE manipulations, including EDTA addition, sodium thiosulphate addition, C18 extraction, aeration, filtration, and graduated pH manipulations. One aliquot of an effluent had brine added prior to the TIE manipulations and the other aliquot had brine added after the TIE manipulations. The timing of brine addition had no effect on the outcome of the toxicity tests with mysids and the authors concluded that it is preferable to add brine as soon as possible after sampling the effluent.

Bleckmann *et al.* (1995) investigated the use of freshwater surrogate species, as well as the Microtox®, for use in TIEs for essentially freshwater effluents which are discharged into estuarine environments. They compared the sensitivity of marine and freshwater test species to a refinery wastewater effluent. The two marine species specified in the effluent permit regulations were more sensitive to the toxicants than were the freshwater species. Interpretations of test results are complicated by factors other than toxicity when essentially freshwater wastewaters flow into estuaries and the effluent permit requires marine organisms for testing. Of the five species tested, mysid shrimp were found to be most sensitive to unidentified toxicants in petroleum refinery wastewater. The sensitivity of the organisms was not correlated with any of the wastewater constituents.

Other effluents, such as cooling waters, may also cause toxicity in a freshwater environment. Fort *et al.* (1995) investigated the toxicity of cooling water effluents to freshwater organisms. The cooling water was obtained from a large freshwater river and then recirculated prior to discharge into the same river. The effluent was consistently toxic to the invertebrate *Daphnia pulex* but caused no toxicity to fathead minnow during 48 h acute tests. A TRE was conducted to investigate the causes of toxicity, to locate potential sources of the suspected toxicant(s), and to identify practical means to reduce toxicity. The by-products of corrosion from the cooling tower were identified as the primary sources of toxicity and a remedy for the toxicity problem, involving reduced recycling and partial dilution, was proposed and implemented.

iii) Metals

Few TIE studies have been conducted with metal containing effluents. However, recent studies have examined the toxicity of solutions containing mixtures of several metals, in particular those associated with irrigation runoff. In most cases, the toxicity of these mixtures is additive (e.g. each metal contributes to the toxic response according to its relative toxicity and its proportional concentration; As, Cd, Cr, Cu, Hg, Ag, V, Zn, Se: Hamilton and Buhl 1990a; B, Mo, Se: Hamilton and Buhl, 1990b).

Somewhat different conclusions were reached in the study of Masnado *et al.* (1995). This study involved testing of a simulated mining effluent in order to verify that the effluent permit specifications for metals would protect the receiving habitat. The test solutions consisted of various matrices of five metals (Cd, Cu, Ni, Zn and Cr) at different hardness levels. The authors developed a model to predict toxicity, based on additive toxicity of mixtures of the metals. However, there was not a good relationship between the predictions of the model and the actual toxicity of the mixture. The authors concluded that effluent limits should be based on actual testing of the combined effects (as opposed to calculating additive effects) and that the use of such synthetic solutions was a useful approach.

Bailey *et al.* (1995) adapted the Environment Canada echinoderm fertilization assay in a partial TIE study involving a municipal effluent. Initial testing revealed that the sand dollar *Dendraster excentricus* was more sensitive than the sea urchin *Strongylocentrotus purpuratus*. The results from the Phase 1 TIE study suggested that a cationic metal was causing toxicity, with copper being the primary candidate. The TIE included additional bioassays involving ammonia and several cations. No-observable-effect concentrations were determined for Ag, Cd, Cu, Hg, as well as ammonia. The authors suggest that the two species differ in sensitivity to copper and ammonia (Bailey *et al.* 1995).

In another marine study, Burgess *et al.* (1997), suggest the use of cation-exchange instead of EDTA addition in marine TIEs. EDTA addition is one of the manipulations used in a Phase I TIE to indicate toxicity caused by divalent metals, yet an alternative approach is the use of cation-exchange chromatography (CEC). The authors compared five cation-exchange media, with the intention of integrating CEC as part of a marine TIE. The solid-phase extraction media in Supelco's LC-WCX and Alltech's Extract-Clean IC-Chelate columns consistently extracted and eluted toxic concentrations of a mixture of metals spiked into seawater, caused no acute toxicity in blank solutions and could be used with environmental samples. The columns were able to remove from 80 to 100% of five metals (Cd, Cu, Ni, Pb, Zn) from spiked seawater, and 85 to 100% (for Cd, Ni, Pb, Zn) could be eluted back into solution. Column breakthrough had to be assessed when environmental samples were used. Column treated samples showed reduced toxicity and decreased metal concentrations, while eluted samples were as toxic as whole samples. The methodology described can be used to assist in the TIE characterisation and identification of toxic metals in environmental samples.

Hockett and Mount (1996) examined the effect of EDTA and thiosulphate additions on the toxicity to *Ceriodaphnia dubia* of solutions containing 16 metals. Thiosulphate addition was more effective than EDTA for reducing the toxicity of Ag and Se, while EDTA was better at removing Zn, Mn, Pb and Ni toxicity. Neither treatment affected Cr, Fe, or Al. The authors suggest that these findings could be used to characterize the metals in unknown samples (Hockett and Mount 1996).

iv) Ammonia

Ammonia is often implicated in the toxicity of several kinds of effluents, as well as other types of samples. For example, Ernst *et al.* (1994) conducted a study of a municipal landfill leachate with the intent to characterise the chemical constituents and relate these to toxicity. While this study was not organized as a TIE, the approach used involved characterisation and identification of suspected toxicants. Initially, samples of leachate were obtained from a municipal waste landfill near Halifax, Nova Scotia and analysed for a range of chemical parameters. Organic chemicals were identified by using mass spectrometry and a reference spectrum library. This chemical characterisation of the samples was accompanied by acute toxicity tests involving rainbow trout (*Oncorhynchus mykiss*), brook trout (*Salvelinus fontinalis*) and an invertebrate (*Daphnia magna*). It is interesting to note that the acute toxicity of the leachates in this study was primarily due to ammonia (Ernst *et al.* 1994). The authors did not present the cost of the study, yet if a TIE protocol had been followed, the cause of toxicity may have been determined in a less costly manner.

Curtis *et al.* (1995) conducted a TIE study for a printed circuit board facility with both fathead minnows and the invertebrate *Daphnia pulex*. The effluent was toxic to both species, yet the TIE indicated that toxicity to the fish and daphnid was due to different causes. Un-ionized ammonia was identified as the primary toxicant for fathead minnow, while an organic compound (a surfactant/defoamer) was implicated in the case of *D. pulex*. The primary cause of toxicity to the daphnids was finally identified as a phosphate ester. In the case of *D. pulex*, the solution to the toxicity problem was product substitution, which was easily accomplished, resulting in a reduction of effluent toxicity to *Daphnia pulex*. However, ammonia toxicity to fathead minnows continues to cause problems and the authors proposed a strategy to partially reduce the ammonia-related toxicity.

The study of Peter *et al.* (1995) is not strictly a TIE study, yet it does deal with testing the effects of different treatments on an effluent. The authors examined the applicability of two assays (an algal chlorophyll fluorescence test with *Scenedesmus subspicatus*) and the Microtox® system to evaluate treatment improvements for a coke plant effluent. Toxicity tests and chemical analyses (dissolved organic carbon (DOC) and ammonia) were conducted on the treated effluents. Toxicity decreased as DOC or ammonia decreased, but the effect of reduced DOC was more important in reducing toxicity. While both bioassays were able to indicate which effluent treatments reduced toxicity, there was a poor correlation between the two (Peter *et al.* 1995).

A new application of the TIE is with pore waters derived from sediments. Vansprang and Janssen (1997) used a modified TIE approach to confirm ammonia toxicity in contaminated sediments from four sites in the Upper Scheldt in Belgium. All of the pore water samples were acutely toxic to the freshwater crustacean *Thamnocephalus platyurus*. Toxicity was removed or reduced by cation exchange resins and air stripping at pH 11. Toxicity was pH dependent, increasing at high pH and decreasing at lower pH. Ammonia was suggested as the main toxic agent and elevated ammonia concentrations were measured in the pore water samples. The modified TIE appears to be a useful tool for the identification and confirmation of toxicants in contaminated sediments.

v) Unidentified Toxicants

Rodgers *et al.* (1996) conducted a "treatment-based" study involving Ontario Hydro radioactive liquid waste. The radioactive liquid waste (RLW) system in Ontario Hydro's pressurised heavy water reactors collects drainage from a variety of sources ranging from floor drains to laundry waste. RLW effluent was intermittently toxic to rainbow trout and *Daphnia magna* during the first phase of Ontario's Municipal Industrial Strategy for Abatement (MISA) Program, apparently as a result of the interaction of a variety of known and unknown organic and inorganic compounds. Accordingly, a treatment-based approach to reducing toxicity was used, supplemented by chemical analysis. Two series of toxicity reduction tests were conducted. The first series explored the potential for sorption of the possible toxicants, and involved use of activated carbon and cation exchange columns. Of the 24 samples in the first test series, 17 were toxic (*D. magna* mortality = 50%). Of the toxic samples, only 7 of 17 were still toxic after passage through an activated carbon column, but 5 of 6 samples tested remained toxic after passage through a cation-exchange column. The second series incorporated a wider variety of treatments, including photo-oxidation, UV oxidation, anion exchange resins, oxygen sparging and passage through a column containing a neutral non-specific resin. In this series, at least one of the treatments was effective in reducing toxicity (16 of 24 samples), but no single treatment was effective for all toxic samples. No relationship between the concentrations of metals and toxicity was found. Since toxicity could be localized to specific streams in each plant, the authors concluded that the toxicity problem may be corrected using a combination of management practices and small scale treatment facilities.

vi) TRE/Treatability Studies

Johnson and Goodfellow (1996) conducted a TRE at one of the U.S. Army's wastewater treatment plants (WWTP). They first assessed the various discharges at the WWTP using Phase I TIE manipulations and toxicity tests involving fathead minnow and *Daphnia pulex*. Six major sources of toxicity were located and primary toxicants were identified. The toxicants, petroleum hydrocarbons and citrus-based cleaning solvents were traced to the biological media of the WWTP trickling filter. The authors undertook a TRE program to identify potential petroleum/citrus-based solvent sources throughout the installation and developed management practices to minimise the potential for their discharge into the sanitary sewer. They focussed on activities which involved large quantities or frequent use of petroleum hydrocarbons or citrus-based solvents. Three areas of primary potential concern were identified for further remediation: fuel purging activities, oil/water separator use, and miscellaneous industrial operations.

Musterman and Flippin (1994) conducted TRE/ treatability studies for a synthetic fibre plant. A series of batch treatability screening tests were conducted to identify technologies suitable for removal of ethylenediamine (EDA) and aquatic toxicity (fathead minnow, *Ceriodaphnia dubia*) in a wastewater discharge from a synthetic fibre plant. Air stripping, cation exchange resin, activated silica, macro reticular resin, granular activated carbon and bio-hydrolysis were evaluated. Only cation exchange resin and bio-hydrolysis reduced effluent toxicity. Bench-scale activated sludge treatability tests were conducted over a four month period under simulated warm and cold weather operating conditions. The results confirmed that activated sludge treatment alone could consistently provide greater than 95 percent BOD reduction and complete EDA hydrolysis, nitrification and toxicity reduction. A reduction in toxicity could be accomplished at high organic loading but hydrolysis of EDA caused the effluent ammonia concentration to increase to toxic levels (>300 mg/L). A lower organic loading was selected to allow for lower levels of ammonia (25 mg/L). Alternatively, the plant could be operated at high organic content level if a process involving the high pH air stripping of ammonia was an option.

Activated sludge plants are a common wastewater treatment method, but they are sensitive to shock loading and their capacity to remove poorly biodegradable or toxic substances is limited. In Finland, Tuhkanen *et al.* (1997) examined the effects of ozonation of pulp mill effluent prior to activated sludge treatment. In this study, pre-ozonation was investigated in order to prevent process failure, to improve the purification efficiency and to reduce the toxicity of the waste water to *Daphnia magna* and to luminescent bacteria (Microtox®). Ozonation increased biodegradability (converting COD to BOD) and resulted in an increase in overall treatment efficiencies. Pre-ozonation prevented process failure caused by bulking of sludge and successfully decreased the acute toxicity to the bacteria and the water flea.

3.1.1 Conclusions of Literature Review

In conclusion, there was little pertinent literature directly related to Canadian mining and TI/RE studies and therefore the application of TI/REs to Canadian mines could not be assessed on this basis. However, the limited number of articles reviewed in this document should not give the impression that there are few evaluations being done. Evidence to the contrary is provided by several sources. These include our contacts with industry, discussions with other consultants, presentations at conferences, and laboratory activities in institutional laboratories in Canada and the U.S. The main conclusion of this review is that the overwhelming majority of TIE studies are never published in the scientific literature for various reasons. This can be unfortunate, since this limits contact and deters the exchange of information which is vital if the field is to advance.

3.2 Survey Results

A total of 119 survey questionnaires were faxed to mine operators across Canada. A total of 53 responses were received. A summary of all raw survey data is provided in Appendix C. The largest number of responses were received from mines in Ontario (40%), followed by Quebec (25%) and British Columbia (15%) (the response was reflective of where the majority of Canadian mines are located) (Table 3-1, Figure 3-1). Of the 53 responses received, 11 were not included in the review. These 11 responses were excluded since the mine either did not have an effluent discharge to a natural watercourse or was not yet in operation. Surveys were received from a wide variety of mine types, including 2 metal refineries (Table 3-2, Figure 3-2). The largest number of responses were received from gold mines (30%), followed by copper/nickel (17%), copper/zinc (12%) and uranium (12%) mines. Note that the presentation of responses according to mine type must not be used for comparisons among mine types (e.g., gold versus copper), since the survey is biased by differences in the percentage of responses received from each mine type. For example, due to their larger representation, gold and copper/nickel mine types reported the largest number of toxic effluents. In comparison, only 2 responses were received from copper mines, both of which were reported as having nonlethal effluents. Yet, this in no way reflects the overall "toxicity status" of gold, copper/nickel or copper mines in Canada. Rather, it was intended that the information presented by mine type be used as a tool for resolving toxicity issues by other mines which fit into a similar category. It should also be noted that several mines did not complete the answers to all of the survey questions. Where possible, the mines were contacted directly to obtain further information.

Of the 42 valid surveys, 17 (41%) mines reported their effluent as being nonlethal, 24 (57%) mines reported having experienced acute toxicity, and only 1 (2.4%) was reported as sublethally toxic (Table 3-3, Figure 3-3). Data from the copper, molybdenum and copper/nickel/cobalt mine types were not included in further analysis since these effluents were reported as being nonlethal. Of the 25 mines that reported having toxic effluents, 9 (36%) reported that toxicity was consistent, compared with 16 (64%) that reported experiencing transient toxicity.

Table 3-1. Summary of responses received according to province

Province	Number of Responses Received
Newfoundland	1
New Brunswick	1
Quebec	13
Ontario	21
Manitoba	2
Saskatchewan	4
Alberta	1
British Columbia	8
Northwest Territories	2
TOTAL	**53**

Table 3-2. Number of responses received according to mine type

Mine Type	Number of Responses Received	# of non lethal effluents	# of acutely lethal effluents	# of sublethally toxic effluents	# of mines reporting consistent toxicity	# of mines reporting transient toxicity
Zinc/lead	3	2	0	1	1	0
Copper	2	2	0	0	0	0
Copper/zinc	5	2	3	0	2	1
Gold	13	7	6	0	3	3
Uranium	5	1	4	0	1	3
Molybdenum	1	1	0	0	0	0
Copper/nickel	7	0	7	0	1	6
Gold/silver/copper	2	1	1	0	0	1
Refineries	2	0	2	0	0	2
Nickel/copper/cobalt	1	1	0	0	0	0
Copper/zinc/lead	1	0	1	0	1	0
TOTAL	**42**	**17**	**24**	**1**	**9**	**16**

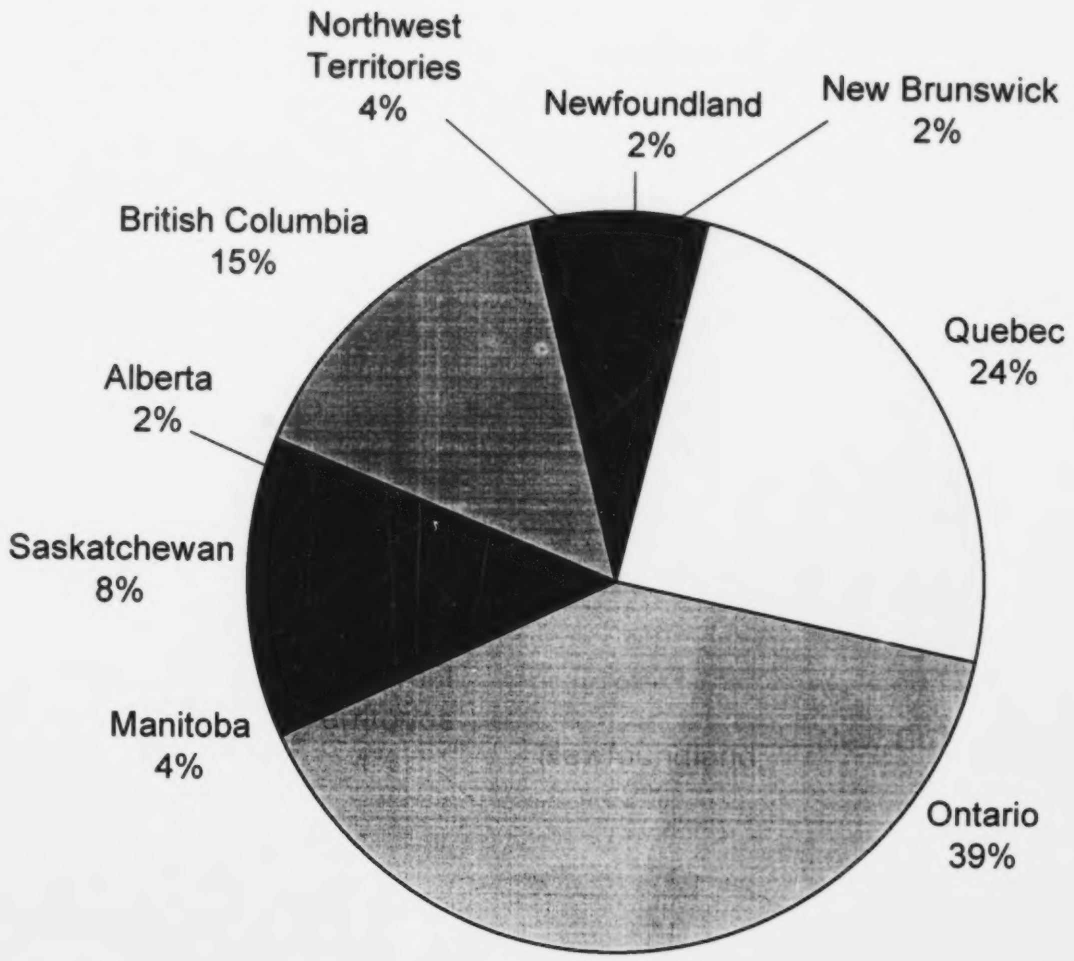

Figure 3-1. Summary of Survey Response by Province

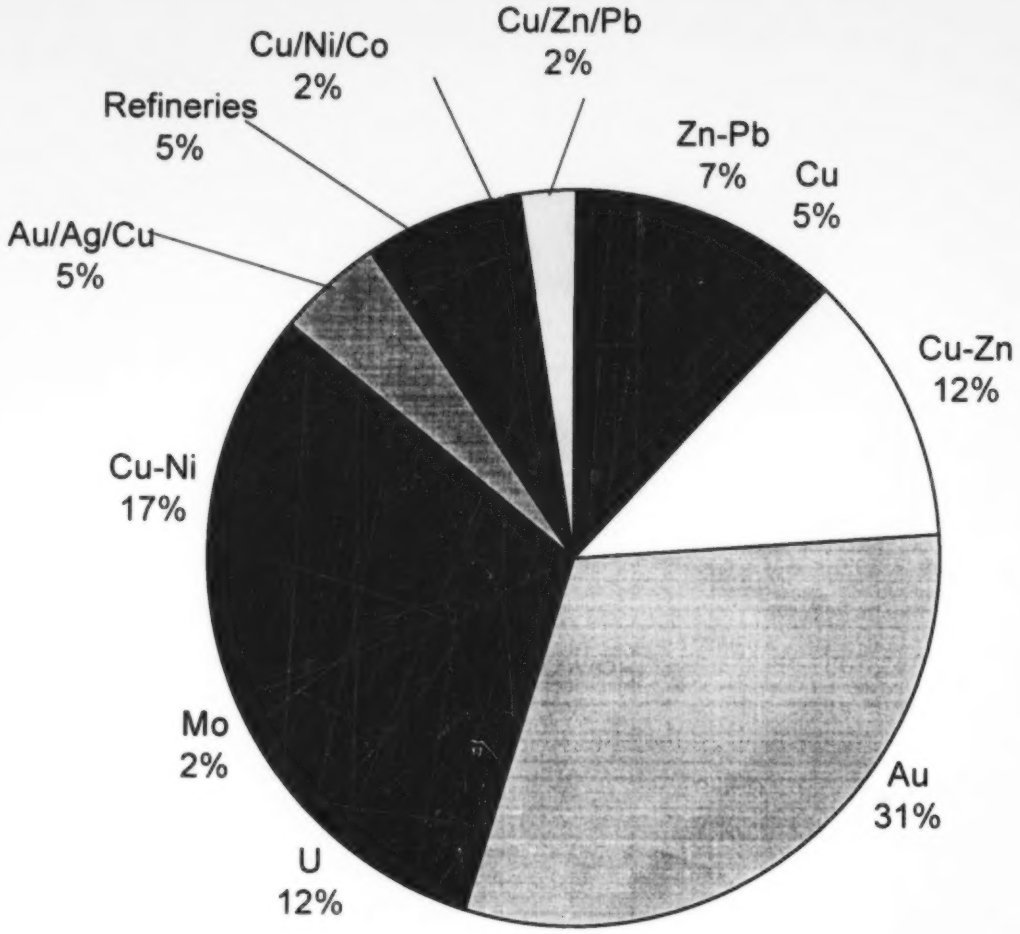

Figure 3-2. Summary of Survey Response by Mine Type

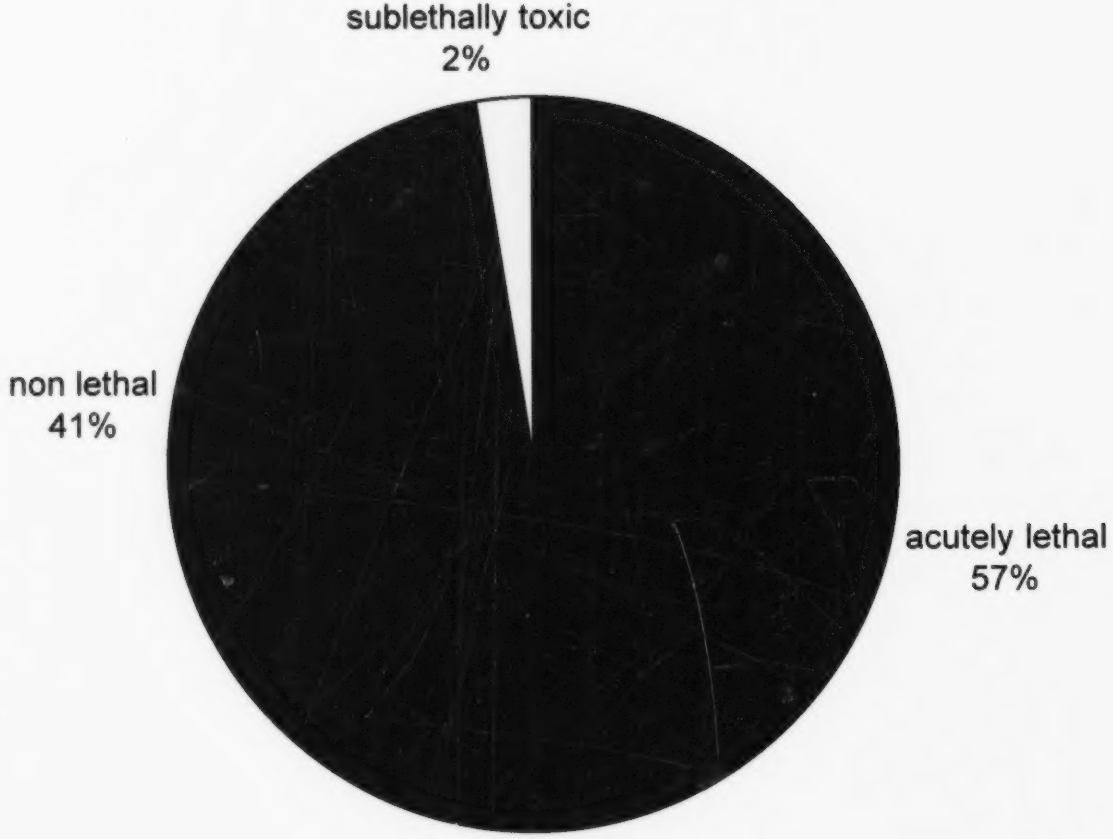

Figure 3-3. Summary of Reported Lethal, Non-Lethal and Sublethally Toxi Effluents

For the 25 mines that reported having toxic effluents (24 lethal and 1 sublethally toxic), 7 (28%) indicated that a TRE had been conducted, while 19 (76%) indicated that at least one TIE study had been initiated (Table 3-3). Of the 19 mines that reported having conducted a TIE study, 16 completed at least one Phase I study and two mines indicated that their Phase I study was still in progress. The remaining mine (uranium - mine #2) indicated that an informal toxicity investigation had been conducted. Five mines reported conducting a Phase II TIE study and 3 completed Phase III. The majority of TIEs were conducted during the 1990's, with many having been initiated between 1993 to 1995. Only 2 were conducted during the late 1980's.

The test organisms most commonly used for the TIE studies were *Daphnia magna* and rainbow trout (Table 3-4). The next most commonly used test organism was the fathead minnow. Four mines had reported using the acute Microtox® test. In contrast to the U.S. EPA TIE protocol which was developed for tests with *Ceriodaphnia* and fathead minnows, *Ceriodaphnia dubia* was one of the least used test organisms.

Table 3-3. TI/RE experience for those mines reporting toxic effluents

Mine Type	# of reported toxic effluents	# mines that have conducted a TRE	# of mines that have conducted a TIE
zinc/lead	1	1	1
copper/zinc [a]	3	2	2
gold	6	1	4
uranium [b]	4	3	3
copper/nickel	7 [c]	0	5
gold/silver/copper [a]	1	0	1
refineries [a]	2	0	2
copper/zinc/lead	1	0	1
TOTAL	**25**	**7**	**19**

[a] TIE for one mine/refinery is still in progress

[b] includes mine that used informal process

[c] TI/RE not conducted for one of the copper/nickel mines since the mine is closing in 1998

Table 3-4. Summary of test organisms used for TIE testing

Test Species	Number of times reported as TIE test organism
Rainbow trout	12
Daphnia magna	14
Ceriodaphnia dubia	2
Fathead minnows	6
Acute Microtox®	4
Algae	1

Of the 17 completed TIE's (including the mine which conducted an informal investigation), only 6 (35%) successfully identified the substance(s) responsible for effluent toxicity (Table 3-5). Ammonia was the most commonly identified cause of effluent toxicity. Toxicity also appeared to be highly pH dependent. For example, elevated pH alone (10.4) was a possible contributor to toxicity in the copper/nickel mine effluent. The copper/zinc/lead mine reported that ammonia was the primary source of toxicity to trout and fathead minnows. However, at lower pH levels, the effluent became toxic to *Daphnia magna* and *Ceriodaphnia dubia*, suggesting the presence of a second unidentified toxicant. Gold mine #1 reported that aeration during the regulatory test caused a rise in pH which resulted in an increase in the concentration of un-ionized ammonia and subsequent trout mortality. The effluent was nonlethal if the sample was refreshed on a daily basis rather than aerated during testing. The uranium mine which identified ammonia as the source of toxicity used an informal approach to toxicant identification, but did not provide further details regarding the methods used. In comparison, ammonia was not one of the commonly encountered mining toxicants in the United States. Conversations with American researchers and consultants revealed that the most commonly identified toxicants associated with metal mining were: heavy metals, total dissolved solids (TDS), chemicals associated with effluent treatment (e.g., flocculants) and fine particulates (possibly associated with metals) (pers. comm. Dave Mount, Russ Hockett 1998).

Copper and a process solvent were also identified as causes of toxicity by a copper/zinc and uranium mine, respectively. In addition, several of those mines which identified a specific toxicant also suspected other possible contributors to toxicity. For example, the copper/nickel mine which identified high pH and ammonia as the source of toxicity, also suspected metals and sulphide as secondary contributors to toxicity (Table 3-5). Further TIE investigations did not identify the secondary toxicant(s).

For the remaining mines, the TIE process did not result in the identification of a specific cause of toxicity and only 7 (41%) reported either the suspected toxicant(s) or the general characteristics of the substance(s) responsible for toxicity (Table 3-6). Three of the copper/nickel mines reported making attempts to conduct a TIE, however, due to transient toxicity the full Phase I TIE was not completed. Consequently, these mines were unable to determine the cause of toxicity or provide information on the characteristics of the substance(s) responsible. One of the refineries reported that in an on-going TIE, the traditional treatments were ineffective at reducing or eliminating toxicity. Although ammonia was not a possible source of toxicity, zeolite was the only treatment that eliminated trout and daphnid mortality. Subsequent treatments with anion and cation exchange resins had no effect on toxicity and the toxicant or the specific characteristics have yet to be identified. Three of the 6 mines which reported not conducting a TIE, even though their effluents were acutely lethal, provided comments regarding their thoughts as to the cause of toxicity. For example, two gold mines suspected metals and ammonia were the source of toxicity, and a copper/nickel mine indicated that toxicity was only observed at high pH levels. Only one mine (zinc/lead) reported having conducted a TI/RE investigation on a sublethally toxic effluent. This mine had observed consistent sublethal toxicity and had conducted a TRE, Phase I and partial Phase II TIE using fathead minnows and *Ceriodaphnia dubia*. The results from the study were inconclusive and further conversations with mine personnel indicated that there appeared to be low reproducibility of the tests and treatments when using sublethal (e.g., IC25) endpoints.

Six mines reported that the TI/RE process took longer than 12 months to complete, and three mines each reported that TI/RE process took between 2-6 and 6-12 months to complete (Table 3-7). Based on the survey responses, the majority of TI/REs cost less than $50,000 to complete. However, two mines reported costs in the range of $50,000 to $100,000 and one mine reported costs exceeding $100,000 (Table 3-8). The zinc/lead mine which conducted a sublethal TI/RE reported that the process took 2-6 months to complete at cost of approximately $75,000. Three mines that reported TI/RE costs of less than $10,000 were those that had made several attempts at conducting TIEs, however, due to transient toxicity the full Phase I TIE was never completed. These mines reported that large volumes of effluent were collected and shipped to the testing laboratory only to find out that the effluent was nonlethal or that toxicity did not persist past 24 hours. Note that not all mines provided cost or timing information in their responses.

Table 3-5. Summary of mines that identified the substance(s) responsible for toxicity

Mine Type	# of mines which successfully identified the substance(s) responsible for toxicity	Substance reported as the cause of effluent toxicity	Additional characteristics of toxicant(s)
zinc/lead	0	-	-
copper/zinc - mine # 1 [a]	1	copper, ammonia	- pH related toxicity; toxicity to *Daphnia* increased at lower pH
gold - mine # 1	1	ammonia	-
uranium - mine # 1	1	solvents	- toxicity decreased during aeration
uranium - mine # 2 [b]	1	ammonia	- pH related toxicity; toxicity reduced at lower pH
copper/nickel - mine # 1	1	high pH, ammonia	- pH related toxicity; toxicity reduced at lower pH; also suspected metals and sulphide as secondary toxicants
gold/silver/copper [a]	0	-	-
refineries	0	-	-
copper/zinc/lead - mine #1	1	ammonia	- pH related toxicity; ammonia was cause of fish toxicity at high pH, but toxicity to *Daphnia* was observed at lower pH (secondary toxicant present)
TOTAL	6	-	-

[a] TIE for one mine is still in progress

[b] used informal process to identify toxicant

Table 3-6. Summary of mines that identified the general characteristics of the suspected toxicant(s)

Mine Type	# of mines that identified general characteristics of suspected toxicant(s)	General characteristics
zinc/lead	0	-
copper/zinc - mine #2	1 [a]	- addition of lime decreased toxicity
gold - mine #2	1	- copper and silver were suspected as causes of toxicity
gold - mine #3	1	- suspected high TDS as cause of toxicity
gold - mine #4	1	- suspected copper and other unidentified substance(s)
uranium [b]	0	-
copper/nickel - mine #2	1	- toxicity was not persistent, ammonia and metals suspected as cause of toxicity
copper/nickel - mine #3	1	- metals and organics suspected as cause of toxicity
gold/silver/copper [a]	0	-
refinery #1	1	- 1993 Phase I indicated toxicity marginal at high pH and increased at near neutral pH, suspected trace metal in 1993; 1996 Phase I indicated toxicity was eliminated with zeolite treatment; suspected metals and TDS as cause of toxicity in 1996
copper/zinc/lead	0	-
TOTAL	7	

[a] TIE for this mine is still in progress

[b] includes mine that used informal process

Table 3-7. Summary of time required to conduct TI/RE studies

Length of TI/RE process	Number of Responses
0 - 2 months	1
2 - 6 months	3
6 - 12 months	3
>12 months	6

Table 3-8. Summary of costs associated with TI/RE studies

Approximate cost of TI/RE process	Number of Responses
≤ $10,000	8
$10,000 - $25,000	1
$25,000 - $50,000	4
$50,000 - $75,000	1
$75,000 - $100,000	1
>$100,000	1

Five mines reported making changes to their treatment system or process as a result of their TIE studies (Table 3-9). The type of changes ranged from product substitution to the building of a full effluent treatment plant. Only 2 mines reported that toxicity was eliminated following the changes and 1 mine reported that toxicity was reduced. Uranium mine #1 was able to eliminate toxicity by substituting a toxic process chemical with one that was assessed to be less toxic. Uranium mine #3 eliminated toxicity by the addition of a primary clarifier and increased settling time. Gold mine #1 identified ammonia as the main cause of toxicity and added CO_2 for pH control, however no change in toxicity was observed. Gold mines #2 and #4 both made changes to their treatment systems costing approximately $5-6 million and $1 million, respectively. Note that neither mine actually identified a specific toxicant during the Phase I TIE and only mine #4 reported a reduction in effluent toxicity following changes to the treatment system. Gold mine #2 did conduct pilot plant treatability studies which indicated that toxicity should have been eliminated after treatment, however the effluent remained toxic after the treatment plant was in full operation. One mine (copper/nickel #3) and one refinery reported that the effluent had been re-routed to an alternate treatment facility with a longer retention time. However, the mine did not feel that the results generated from TIE studies were a major factor in the decision to re-route the effluent. The estimated cost associated with re-routing one of the effluent streams was approximately $4 million. Four copper/nickel mines also reported that pH adjustment was installed as part of a provincial regulation and not as a result of the TIE studies.

Of the 17 completed TI/REs (including the mine which conducted an informal investigation), only 5 (29%) reported that the results were worth the cost of the TIE. Satisfaction with the TI/RE process was related to either identification of a specific toxicant or elimination of toxicity. For example, four of the five mines that were satisfied with the TI/RE process had identified a specific toxicant as the cause of toxicity. The fifth mine (uranium mine #3) did not identify a toxicant, but was able to eliminate toxicity after the addition of effluent treatment (Table 3-10). In comparison, two mines (copper/nickel - mine #1 and copper/zinc/lead - mine #1) reported that a specific toxicant was identified (ammonia), but did not believe the results generated from the TI/RE studies were worth the costs incurred. In both cases, toxicity was reported to be pH dependent, and neither mine was able to identify the secondary causes of toxicity. The copper/zinc/lead mine reported to have investigated a variety of possible treatment options for ammonia, including break point chlorination, stripping towers and biological treatment. However, it was concluded that there were no economical technologies available to treat for ammonia in a northern climate.

Table 3-9. Summary of mines that implemented changes to effluent treatment or process following TIE studies

Mine Type	# of mines that made changes to effluent treatment or process	Cost of Change	Description of Change	Outcome
gold - mine #1	1	not provided	Added CO_2 for pH control	no change in toxicity
gold - mine #2	1	$5-6 million	Built effluent treatment plant, including increased settling time, INCO SO_2/air process and pH control	no change in toxicity
gold - mine #4	1	approx. $1 million	Added INCO SO_2/air process	reduction in toxicity
uranium - mine #1	1	not evaluated	Chemical substitution	toxicity was eliminated
uranium - mine #3 [a]	1	not provided	Added primary clarifier and increased settling time	toxicity was eliminated
TOTAL	**5**			

[a] mine did not identify toxicant(s) and did not provide characteristics of toxicant(s)

Table 3-10. Summary of mines reporting that results from the TI/RE process were worth the cost

Mine Type	Substance reported as the cause of effluent toxicity	Approximate Cost of TIE	Changes to treatment or process (Yes/No)	Outcome
copper/zinc - mine # 1	copper, ammonia	$28,000	No - mine closed	effluent still occasionally toxic
gold - mine # 1	ammonia	$6000	No - technology to treat ammonia not available	effluent still occasionally toxic
uranium - mine # 1	solvents	$30,000	Yes	toxicity was eliminated
uranium - mine # 2 [a]	ammonia	not provided	No - mine closed	information not provided
uranium - mine # 3	not identified	not provided	Yes	toxicity was eliminated

[a] used informal process to identify toxicant

A complete assessment of the Canadian mining sector's experience with the TI/RE process was not possible since less than 50% of mines responded to the survey. Of the 17 mines which reported having completed a TI/RE, only 6 (35%) successfully identified the substance(s) responsible for effluent toxicity and only 5 (29%) reported that the results were worth the cost of the TIE. Based on the survey responses, there are several possible reasons as to why the TI/RE process did not result in the identification of a specific cause of effluent toxicity including:

- transient or non-persistent effluent toxicity,
- presence of complex multiple toxicants,
- lack of communication between the mine and testing laboratory,
- failure to continue the investigation to the next phase due to cost considerations and
- a lack of experience by the laboratory conducting the TI/RE study.

As evident from the survey responses, TI/REs were not successful for those effluents that were sporadically toxic or when toxicity dissipated over time. However, the degree of effluent variation and applicability of the TI/RE processes are often not known until a detailed toxicity study is actually initiated. For example, based on only 1 yearly test with rainbow trout and *Daphnia magna*, two respondents concluded that their effluents were nonlethal. Several mines also reported attempts at Phase I TIEs on samples that were often found to be nonlethal or in which toxicity did not persist past the initial untreated test. Yet information on the stability of the toxic response can be very useful in determining the cause of effluent toxicity (e.g., the toxicant may have been volatile, or may have precipitated reducing bioavailability). However, the U.S. EPA guidance clearly indicates that TIEs require that toxicity be present frequently enough and endure storage (that is, the toxicity is not rapidly degrading) so that repeated testing can characterize and subsequently identify and confirm the toxicants in Phases II and III. Therefore, enough testing should be done to assure consistent presence of toxicity **before** TIEs are initiated. This is not done to validate a given test, but to establish the sufficient and frequent presence of toxicity (U.S. EPA 1991a). For a TIE study to be successful when toxicity is not persistent, treatments and manipulations will often have to be conducted as soon as the sample is received rather than after the untreated test is initiated. However, the decision to proceed with this testing approach should be based on a sufficient amount of historical toxicity data indicating the frequency and persistency of effluent toxicity. Alternatively, the toxicity treatability path may be a more useful approach since it is known that the toxicant(s) can be removed (e.g., the toxicant is non-persistent). In cases where toxicity is sporadic, prior to conducting the TI/RE, it may be necessary for mine and laboratory personnel to investigate the use of an on-site indicator to predict when the effluent may be toxic. For example, effluent toxicity may be associated with certain effluent parameters (e.g., high pH or conductivity) or a particular mine process. While their usefulness will likely be mine-specific, on-site screening tests (e.g., Microtox®, *Daphnia* IQ) may also be effective tools for predicting effluent toxicity when a significant correlation can be established between the TIE test species and the selected screening test.

The success of a TI/RE is also dependent on the participation of mine personnel in the study. To quote one of the survey respondents, "the lack of understanding by the two groups, TIE specialists and mine personnel, of each other's processes can lead to the incomplete transfer of information". Mine personnel must be willing to actively participate in the study by providing process information as well as historical toxicity and chemistry data to the laboratory conducting the study. The mine should also insist on a detailed study plan which describes the treatments to be used during the study. The success of a TI/RE study can also be greatly reduced if mine processes are constantly changing and the toxicants vary greatly from one sample to the next. In these instances, it is critical that process changes at the mine site are well documented and the information conveyed to the laboratory conducting the investigation.

The TI/RE process outlined by the U.S. EPA was originally published in 1991 and updated in 1993. Since then, the investigative techniques have evolved and the success or failure of the TIE often depend on the experience of the laboratory conducting the study. To quote one of the respondents, the knowledge and experience of the investigator is critical if you are to get beyond the "speculating stage". Comments from researchers and consultants experienced with the TI/RE process as applied to mining effluents in the United States clearly support this statement. While the majority of laboratories are comfortable with conducing a Phase I TIE, many are inexperienced in the design of studies to test a hypothesis that a specific substance(s) is the cause of effluent toxicity (pers. comm. D. Mount, U.S. EPA, Duluth Laboratory). This may explain why few mines reported testing beyond Phase I. It must also be recognized that beyond the Phase I TIE, the approach is not standardized and subsequent studies to identify the specific toxicant require experienced personnel. Laboratories will also often focus on treatments that have both reduced and eliminated toxicity. However, the toxicity characterization treatments which produced the most dramatic results (e.g., those which eliminated toxicity) should be the focus of further investigations. Those treatments which only reduced toxicity but may indicate the presence of secondary toxicants, should be left for later examination (Norberg-King 1997). For example, one mine reported that they were getting better return on investment when the study concentrated on using single concentration tests with those treatments where very obvious reductions in toxicity were observed.

There is also a common misconception that a TIE study will conclusively identify the substance(s) responsible for effluent toxicity. The mine client must understand that TIEs do not "prove" the cause of toxicity, but rather use a weight of evidence approach. Toxicants are often identified by the absence of contrary evidence. For example, test interpretations are generally: "the result is what one would expect from chemical x" or "the result is inconsistent with chemical x" (Mount 1997). The use of traditional statistics in TIE studies can also be of limited value and even misleading. For example, many laboratories will automatically use simple toxicity/concentration correlations to determine if substances x and y are the cause of toxicity. However, this method is not likely to be appropriate for matrix dependent toxicants (e.g., metals) (Mount, 1997). More complex multiple regressions are likely to yield better results for matrix dependent toxicants or in cases where multiple toxicants are suspected. The generation of a sufficient amount of data to provide strong evidence regarding the identification of the toxicant is critical if the mine is to consider investment in costly plant-scale remedial measures.

3.2.1 Summary of Mine Survey Responses

- A complete assessment of the Canadian mining sector's experience with the TI/RE process was not possible since less than 50% of mines responded to the survey.

- Of 42 mines which responded, 25 (57%) reported having experienced acute toxicity.

- Of those mines reporting toxic effluents, 7 (28%) indicated that a TRE had been conducted and 17 (76%) reported having conducted at least one Phase I TIE. Very few mines reported going beyond the Phase I toxicity characterization.

- Of the 25 mines that reported their effluents as being toxic, 9 (36%) reported that toxicity was consistent, compared with 16 (64%) that experienced transient toxicity. Only 1 mine reported having conducted a sublethal TI/RE.

- Of the 17 completed TIE's, only 6 (35%) successfully identified the substance(s) responsible for effluent toxicity.

- Ammonia was the most commonly identified cause of effluent toxicity and toxicity also appeared to be highly pH dependent.

- Several mines reported that the TI/RE studies were unable to identify secondary toxicants.

- TIEs were not successful when toxicity was transient or not persistent.

- Costs associated with TIE studies were generally less than $50,000, but one mine did report spending >$100,000.

- Six mines reported that the TI/RE process took longer than 12 months to complete and six reported the process took between 2 and 12 months to complete.

- Five mines reported making changes to their treatment system or process based on the results of the TI/RE. Two mines reported that toxicity was eliminated following the changes, 1 reported that toxicity was reduced and 2 reported no change in toxicity.

- Changes varied from product substitution to implementation of a full-scale effluent treatment facility.

- Of the 17 completed TI/REs, only 5 (29%) reported that the results were worth the cost of the TIE. Satisfaction with the TI/RE process was related to either identification of a specific toxicant or elimination of toxicity.

3.3 Case Studies

Five cases studies were selected for review. However, it was evident from the survey responses that the reported TI/RE results were very specific to each completed study, and therefore did not completely match the pre-selected categories outlined in the methodology section. Since varying degrees of "success" or "non success" were observed in different parts of each reported study, a brief description of each selected case study is provided below, followed by the presentation of each detailed case study.

Case Study #1 *copper/zinc mine*

- primary toxicants were identified, but also suspected the presence of secondary toxicants
- mine closed, but effluent continues to be discharged and is occasionally toxic
- mine felt TI/RE process was useful

Case Study #2 *uranium mine*

- primary toxicant identified
- modifications were made to process (e.g., product substitution) and toxicity of the effluent was eliminated
- mine felt TI/RE process was useful

Case Study #3 *copper/nickel mine*

- primary toxicant identified, but secondary toxicant(s) were not
- pH adjustment added to treatment, toxicity reduced
- mine did not feel TI/RE process was useful

Case Study #4 *gold mine*

- general characteristics of suspected toxicant(s) identified
- full on-site treatment plant built, but final effluent is still toxic
- mine did not feel TI/RE process was useful

Case Study #5 *cobalt/precious metals refinery*

- general characteristics of suspected toxicant(s) identified
- final effluent is still toxic and investigations are on-going
- mine did not feel the "standard" TI/RE process was useful; complex nature of effluent required development of novel methods and techniques to identify toxicant(s)

The purpose of the case studies was to summarize and comment on the Canadian mining experience with the TI/RE process and not to criticize the abilities of the consultant or researcher conducting the studies. Furthermore, the discussions that occurred between mine personnel and the consultant/researcher conducting the study could not be included in the review and certain "insider" information was therefore undoubtedly lost.

3.3.1 Case Study #1 - TIE with Effluent from a Copper/Zinc Mine

The first case study involves a copper/zinc mine. The mine effluent is treated with lime to increase pH and is discharged into a lake. The effluent was transiently toxic to rainbow trout and *Daphnia magna* and the company engaged a consultant to investigate the cause(s) of toxicity.

The mine was closed in 1992/1993, but continues to treat and discharge effluent. The treated mine effluent is continuously discharged to a lake, where the contaminants settle at lower depths. Since the TIE study was conducted in 1990/91, the treatment system, a high density sludge treatment, was substantially upgraded to optimize operations. The treatment system includes the addition of lime to reduce suspended solids. As part of the improvements, the volume and capacity of the clarifier/settling pond was expanded and pH control (e.g., addition of lime) was improved. These improvements were undertaken without any reference to the results of the TIE study. However, according to an environmental scientist at the mine, similar improvements would have been implemented if the TIE study results had been used.

The mine water originates from a tailings pond prior to treatment. Ammonia levels in the tailings drainage are elevated due to past use of explosives and the treated effluent continues to be sporadically toxic. The control of ammonia toxicity through control of both temperature and pH is not possible with available technology. In addition, the present treatment system increases pH (and hence NH_3). However, since the mine's closure, levels of ammonia have decreased by approximately 50%, and are expected to continue to decrease over time since the mine has ceased operations.

An environmental specialist associated with the mining company responded to the AETE TIE questionnaire and was interviewed over the telephone. He provided the consultant's report on the copper/zinc mine's TIE. After reviewing the report, this investigation was considered a suitable candidate for a case study. Despite the closure of the mine, the study is an appropriate selection since it was conducted fairly recently and since the effluent continues to be released. The study was selected after the approval of the company scientist, who also provided further information regarding the current status of the effluent and treatment.

The TIE study was conducted by a consultant/commercial laboratory firm. Testing involved fathead minnows and *Daphnia magna* in a partial Phase I TIE. Additional toxicity tests investigated the toxicity of ammonia and the metals Cu, Al, Ag in reconstituted water.

The TIE investigation was conducted over a period of one year. The causes of toxicity were identified, at least to the satisfaction of the client. In the opinion of the mine's senior scientist, the results of the TIE were worth the cost of the study.

3.3.1.1 Overview of the TIE Study

The consultant began the TIE process with a review of the historical chemical data and effluent toxicity data. This was a logical step since the toxicity, while transient (i.e., not present in consecutive samples), did occur often enough in several samples to be considered a regular occurrence. By comparing levels of ammonia and metals, the consultants distinguished likely candidates for toxicity. There was no evaluation of operational/ maintenance practices and treatment facilities. However, it was known that the mine was closing and the consultant was aware that the treatment method involved the addition of lime to control pH.

In 1990 the effluent was analysed for levels of metals (Ag, Al, As, Cd, Cu, Fe, Mn, Mg, Na, Ni, K, Pb, Sr and Zn), TDS, Cl, SO_4, ammonia, cyanide, pH, hardness and alkalinity. The report showed mean values of these parameters, determined for a nine-month period and data from three of the months was presented in the table. In general the effluent was alkaline (pH 9.0-9.5), with elevated levels of hardness (~2,500 mg/L), dissolved solids (~4,500 mg/L TDS) and ammonia (~50 mg/L). Note that the report lists Na, Mg, K as charged ions, though it is evident that only the dissolved metals were analysed. (Due to the elevated ionic strength, it is possible that some proportion of these metals were present as bound complexes in the effluent).

The report mentions that four toxicity tests were conducted between January 1989 and September 1990, prior to the TIE study. The effluent was toxic to both trout and *Daphnia* on all four occasions (LC50s < 25% v/v of effluent). The effluent concentrations for LC50s were prepared using laboratory water as dilution water.

The consultant compared levels of components identified in the effluent with values for acute toxicity (generally LC50s) reported in the literature. The concentration of total ammonia was 10 to 100 times (~50 - 60 mg/L) above reported LC50 values for trout and *Daphnia*. Copper was elevated in some effluent samples (0.11 - 0.13 mg/L), and the single reported analysis of thiocyanate, 25 mg/L, was roughly 10 times the LC50 for *Daphnia*. The literature values were obtained from recent references (most dating 1985+).

Comparisons of effluent chemistry data to literature LC50 values indicated that the cause of the toxicity could not be conclusively identified. The reasons given by the consultant were: a limited amount of effluent chemistry data (some parameters were not analysed, not all measured parameters were analysed frequently); a lack of toxicity data for effluent constituents such as dissolved solids; and the inability of chemical analyses to distinguish the toxic forms of certain components (such as non-complexed metals).

The consultant then proceeded with the TIE study, a modified Phase I study to characterize the toxicant. The Phase I TIE included pH adjustment to 3 and 10; filtration at the initial pH, pH 3 and 10; aeration at the initial pH, at pH 3 and 10; treatment with EDTA and treatment with sodium thiosulphate. The Phase I TIE included use of the non-specific absorbent zeolite (incorrectly referred to as cationic exchange in the report) and extraction of neutral non-polar compounds with a C18 column. Data for a series of graduated pH exposures (pH 6 to pH 9) were presented in two tables and five figures in the report, but were not discussed. Baseline tests were conducted so that responses observed in the effluent treatments (e.g., following the manipulations) were compared to the toxicity of an untreated sample.

The main concern of the client, the copper/zinc mine, was effluent toxicity to rainbow trout. The consultant considered that to conduct a TIE with rainbow trout would not be practical. Rainbow trout tests require significant volumes of exposure solutions and the preparation of these solutions in TIE studies would be expensive and impractical. The consultant stated that their laboratory had a large data set showing that fathead minnows had a similar sensitivity to trout for many toxicants. This data set was not provided in the report. The consultant persuaded the mine to proceed with the TIE using juvenile fathead minnows (ranging in age from <24 h to 4 weeks old). Neonate (<24 h old) *Daphnia magna* were involved in initial testing and also in the modified Phase I testing.

Several exposures to the treated effluent samples were single concentration tests and the results were reported as lethal times (LT50s). The baseline tests, and some effluent treatments (sample aeration at pH 11), involved dilutions of the effluent and were reported as LC50s. It is unclear why some treatments involved LC50s, while others did not. The data that appears in the report suggests that the effects of treatments would have been evident if only 100% v/v effluent exposures had been employed. The LC50s obtained in different treatments were not compared statistically, so only extreme increases or decreases in LC50s were considered important. It is probable that similar conclusions regarding the results of treatments could have been obtained from single concentration tests.

The report provided by the mining company does not include the data used to calculate the LT50s and LC50s cited. The consulting company provided sample raw data sheets for selected toxicity tests. Test reports were not issued since the data were summarized in the report. While it was not possible to review all of the raw data, those from the selected toxicity tests corroborated the reported results.

The results of the TIE Phase I study are summarized in Tables 3-11 (fathead minnows) and 3-12 (*Daphnia magna*).

Conclusions of Phase I

The consultant concluded that ammonia was the prime substance responsible for the toxicity to fathead minnow, based on the following:

- ammonia measurements in TIE samples were elevated
- toxicity was reduced following treatment with zeolite
- toxicity was at least partially reduced following pH adjustment to pH 11 and aeration ("stripping" treatment).

The consultant indicated that aluminum may also be implicated in toxicity, since they considered that the levels of the metal (0.48 to 0.86 mg/L) were high enough to cause toxicity to the fathead minnow at pH 8. Al levels decreased after the stripping treatment in August, which also reduced toxicity.

For the limited evaluation of toxicity to *Daphnia*, the consultant concluded that copper was the primary constituent causing toxicity to the invertebrate. This was based on the following results:

- copper measurements in the August sample were elevated (to marginally lethal levels for *Daphnia* according to literature values)
- toxicity was greater at pH 6 than at pH 7 or 8
- toxicity was reduced by adjustment to pH 10
- toxicity was reduced by filtration at pH 8
- toxicity was reduced by addition of EDTA
- toxicity was reduced by addition of thiosulphate
- copper levels were reduced in treatments involving pH adjustment (to pH 10) and filtration

A series of tests was then conducted using reconstituted water that resembled the ionic strength of the effluent, which was separately spiked with ammonia and with metals including Cu, Al or Ag. The simulated effluent was toxic to rainbow trout, fathead minnows and *Daphnia magna.*

Toxicity was reduced by the addition of EDTA, by the addition of thiosulphate and by pH adjustment (to pH 10) and filtration.

However, the toxicity of some samples fluctuated over time. Dissolved copper levels measured in filtered and unfiltered effluent samples were similar. The consultant suggested that other substances, such as ammonia, may be implicated, or copper is undergoing reactions which decrease its bioavailability (and toxicity). In addition, the consultant proposed that silver levels in the effluent may occasionally contribute to toxicity. Finally, mortalities were observed in the controls of some exposures to the simulated effluent (reconstituted water without the addition of metals or ammonia). The consultant suggested that these mortalities may be related to the high levels of TDS present in the effluent. Elevated TDS has been shown to cause toxicity in other types of effluents (Mount *et al.* 1997).

3.3.1.2 Overview of the TIE Study - Experiments with Simulated Effluent

The consultant conducted a series of experiments which would be considered part of a Phase III TIE. These tests involved the use of a simulated effluent, prepared by adding salts to deionized water. Toxicity tests were conducted with ammonia, copper, silver and aluminum at pH 7, 8 and 9, which were separately added to the exposure vessels. Tests were conducted at various pH to evaluate treatment systems to control pH. Testing involved rainbow trout, fathead minnows and *Daphnia magna*, according to Environment Canada test methods. With the exception of some of the assays conducted with ammonia, all of the exposure concentrations were chemically analysed.

Ammonia: The testing indicated that larval fathead minnow and trout were about equally sensitive to ammonia. Since *Daphnia* were five times more tolerant than trout, the elimination of ammonia toxicity to fish would prevent ammonia toxicity to *Daphnia*. The consultant indicated that the LC50s determined with the simulated effluent were similar to literature values determined in waters with lower dissolved solids and lower hardness.

Copper: The results of duplicate tests with copper (LC50s) varied considerably, for fathead minnows and for *Daphnia*. In many cases, tests conducted at the same pH but at different times resulted in decreased toxicity (significantly higher LC50s). The consultant suggests that the elevated TDS of these solutions may have been the cause of this variability.

Aluminum: The toxicity results varied by more than an order of magnitude at pH 9 (a pH where most Al is soluble). The LC50s for Al at pH 7 (where the solubility of Al is minimal), and pH 8 were greater than the highest concentration tested. However, the consultant reported that fathead minnow and trout were about equally sensitive to aluminum.

While the results of toxicity tests with the metals are too variable for precise predictions, the consultant did recommend levels of ammonia which would not cause toxicity to trout. Meeting the acute lethality legal requirements was the main concern of the mine.

3.3.1.3 Comments on the TIE Approach

i) Approach to Phase I TIE

The consultant followed a modified approach to a Phase I TIE, incorporating the use of zeolite at the first stage of testing with fathead minnows. The inclusion of zeolite at this stage was logical, since the review of effluent chemistry provided a good indication that ammonia was a prime candidate causing toxicity. However, zeolite was not used in further testing, nor was it used in testing with *Daphnia*. This is puzzling since ammonia may have been partially involved in the toxicity to *Daphnia*. The removal of ammonia in tests with *Daphnia* may have clarified the contributions of the other suspected toxicant (copper).

Samples of effluent should have been analysed before and after treatment with zeolite. Since zeolite is a non-specific absorbent, other substances, metals in particular, are also removed. While ammonia levels were measured in samples before and after the air stripping treatment, no chemical analyses were done on samples which had undergone zeolite treatment. This would have strengthened the link between ammonia concentration and effluent toxicity for fathead minnow and *Daphnia*.

The results for Phase I treatments involving the graduated pH testing were part of the analyses conducted with the first sample and the results appeared in the figures. Ammonia toxicity may be reduced or eliminated at low pH, yet the results of these graduated pH tests were not discussed in the report.

The usual Phase I approach, as outlined by the U.S. EPA, involves a battery of treatments. The implication is that these treatments allow the effects of substances with different physical-chemical properties to be somewhat isolated. The copper/zinc mine study did not involve all of these treatments and no reason is offered as to why some were eliminated.

In the case of the mine's effluent and trout toxicity, levels of ammonia and metals were obvious candidates for investigation. Thus, since some treatments were eliminated, one would expect that the selection would focus either on treatments that would reduce ammonia and metal toxicity, or treatments that would differentiate between ammonia and metal toxicity (viz., selection of zeolite). Based on the review of effluent chemistry at the beginning of the study, it is unclear why the C18 column treatment was included, while cation/anion exchange was not. The C18 procedure would be expected to remove non-polar organics, yet there is no mention of this class of compounds in the review of effluent chemistry. Passage of the effluent through cation and anion exchange columns would remove metals such as Cu (a cation at pH 8.5) and Al (an anion at pH 8.5). Since copper and other metals were initially suspected as contributing to toxicity, it would have been logical to include the use of cation/anion exchange columns in the Phase I process.

If the Phase I process is modified, it is possible to overlook classes of compounds that contribute to toxicity. However, a knowledge of effluent chemistry and of the industry sector can eliminate some Phase I treatments which are unlikely to affect the outcome, reducing costs and effort. The main critique of this study is not that the Phase I process was modified, but that the logic behind the modifications is not evident. This lack of coherence is due to the fact that explanations for the choice of treatments are lacking, while apparently logical treatments such as cation/anion exchange were not included.

ii) Use of fathead minnows

The rainbow trout is an important regulatory species in Canada, yet fathead minnows were used in this TIE study. As stated by the consultant, conducting a Phase I study using rainbow trout would be impractical. While not explicitly stated in the report, the use of rainbow trout in a Phase I study would necessitate much greater effort and expense since trout require large test volumes. However, it is possible to conduct a modified Phase I TIE with rainbow trout, if there is enough background material to indicate the cause(s) of toxicity. This appears to be the case for this study, since from the beginning, ammonia was strongly suspected of being the main cause of toxicity. Note that the consultant was able to show that the two species had similar sensitivities to ammonia in simulated effluent. However, it appears that there was sufficient background information to limit the number of treatments and permit the use of rainbow trout. This would have been of more value to the client in the Canadian context.

iii) Variation in results

There are variations/contradictions in the toxicity data which are not always explained. For example, a sample was collected on August 8, 1991 and tested on August 16 and August 20. Levels of total and un-ionized ammonia were similar in two untreated effluent samples. However, despite the similarity in ammonia levels, the August 20 sample was less toxic (LC50s of 57/50 % v/v vs LC50 of 92% v/v). The explanation offered is that ammonia was involved in some unspecified chemical reactions which affected the quantity of uncomplexed ammonia. It is unclear what is meant by this.

Similarly, the consultant suggested that copper is the main toxicant for *Daphnia magna*. However, while the March and May effluent samples contained similar levels of copper (0.26 and

0.21 mg/L respectively), the March sample was less toxic (LC50s of 71/85% v/v compared to LC50s of 31/<25% v/v). No explanation is offered for the inconsistency.

iv) Consistency of treatments

There were some inconsistencies noted in the pH adjustments of effluent samples. For example, during the aeration treatment, some samples were adjusted to pH 10, while later samples were adjusted to pH 11. The extreme pH treatments recommended by the U.S. EPA Phase I procedure are pH 3 and pH 11 (followed by aeration). An adjustment to pH 10 is thus non-standard, but was not clearly explained in the report.

By adjusting pH, the ratio of ionized to un-ionized forms of ammonia changes. As pH increases, the proportion of the most toxic form of ammonia (un-ionized ammonia) increases. The un-ionized ammonia can be driven from solution and eliminated by aeration, the treatment applied in this study. At 20°C and pH 10, approximately 80% of the total ammonia is un-ionized, compared to approximately 97.5% at pH 11. There is a significant difference in the proportion of un-ionized ammonia at pH 10 - the lower proportion of un-ionized ammonia may have decreased the efficiency of the aeration treatment at pH 10.

v) Lack of statistics

The report does not include a description of how the LC50s and LT50s were calculated, nor is there any mention of confidence limits for these parameters. Most of the tests were conducted in duplicate, yet the replicate results are not compared statistically, and in some cases, the data were apparently pooled without any statistical comparisons. The additional effort to conduct duplicate exposures is wasted if the data are not properly used. It would have been simpler and less expensive to conduct single exposures with a larger numbers of fish in a larger volume of sample. The lack of statistical comparisons may not be critical in most TIE work, where gross changes in toxicity are the only consideration. Detailed statistical comparisons are often less useful in Phase I or Phase II TIEs. However, this study covered several samples over the course of a year, and all samples were tested for several days in a row. In addition, the report mentions that toxicity was reduced "slightly" after certain treatments. It would be preferable if the data had been compared statistically to account for the variation in effluent and sample toxicity over time and to allow a more rigorous definition of "slight" reductions in toxicity.

vi) Experiments with Simulated Effluent

This study contributed little to the conclusions of the report. The results of the experiments with metals are so variable (replicate LC50s differ by 10 to 100 times) as to be meaningless. The consultant concedes that the ammonia LC50s generally agree with values in the literature. Since ammonia toxicity is influenced by pH and temperature, but is not affected by water hardness, it is unclear why this study with simulated effluent was undertaken. Most of the information concerning ammonia was already available in the literature and the metal toxicity experiments were poorly planned (resulting in extreme variation in the results). The only benefit to this study was that it indicated that both trout and fathead minnow were equally sensitive to ammonia. However, it would have been preferable to demonstrate this at the beginning of the study, and not its conclusion. If differences in sensitivity had been observed, it would have been extremely difficult to account for this at the end of the study, putting at risk the conclusions and applicability of the results.

3.3.1.4 Overall Summary of Case Study

- The client, a copper/zinc mine, was satisfied with the results of the study.
- The consultant conducted a review of effluent chemical data and compared effluent parameters.
- At the conclusion of the study, the causes of toxicity, Cu and NH_3, were identified to the satisfaction of the client. The report identified reduction targets for copper and ammonia to eliminate trout and daphnid toxicity
- Other possible toxicants (Ag, Al and TDS) were also identified.

- A modified Phase I study was conducted, but the choice of treatments was not explained. Some Phase I treatments were not necessary if ammonia and metals were causing toxicity. Other treatments which were not selected would have been useful to detect toxicity due to metals.

- The Phase III study was poorly designed, leading to generation of data of limited value.

- The report identified a strategy for reduction of ammonia toxicity to rainbow trout, the main concern of the client.

Table 3-11. Responses of fathead minnows during Phase I TIE involving effluent from a copper/zinc mine

Treatment	Date Sample Collected				
	Jan 25/91	Mar 1/91	April 5/91	May 6/91	Aug 8/91
Baseline tests	Toxicity increased over 5 d	Toxicity increased over 4 d	Not toxic	Not toxic	Toxicity variable over 4 d
graduated pH (pH 6)		Not toxic			Not toxic
graduated pH (pH 7)		Not toxic			Not toxic
graduated pH (pH 8)		Increased toxicity			Increased toxicity
adjust to pH 3	No reduction				
adjust to pH 10	No reduction				
aeration at pH 3	No reduction				
aeration at pH 8.5/8.3	No reduction				
aeration at pH 10/11	No reduction	Toxicity reduced			Partial reduction
filtration at pH 3	No reduction				
filtration at pH 8.5/8.3	No reduction				
filtration at pH 10/11	No reduction				
C18 column	No reduction				
EDTA	No reduction	No reduction			No reduction
Thiosulfate	No reduction	No reduction			No reduction
Zeolite	Toxicity reduced	Toxicity reduced			
Chemical analyses	yes	Yes	yes	yes	yes

Table 3-12. Responses of *Daphnia magna* during Phase I TIE involving effluent from a copper/zinc mine

Treatment	Date Sample Collected			
	March 1/91	April 5/91	May 6/91	Aug 8/91
Baseline tests at Effluent pH	toxicity decreased over 4 d	toxicity increased slightly over 5 d	toxicity variable over 6 d	toxicity decreased over 4 d
graduated pH (pH 6)				>toxicity than at pH 6 or 7
graduated pH (pH 7)				toxic
graduated pH (pH 8)				toxic
adjust to pH 3			no reduction	
adjust to pH 10			toxicity reduced	
aeration at pH 3				
aeration at pH 8.5/8.3				
aeration at pH 10/pH 11				
filtration at pH 3			slight reduction	
filtration at pH 8.5/8.3			toxicity reduced	
filtration at pH 10/pH 11			no reduction	
C18 column				
EDTA			toxicity reduced	
Thiosulfate			toxicity reduced	
Zeolite				

3.3.2 Case Study #2 - TI/RE with Effluent from a Uranium Mine

The second case study comprises a uranium mine and milling effluent. The effluent is treated in the mill before discharge. The effluent was historically non toxic but several toxicity tests with trout resulted in failures in 1994. The company therefore engaged a consultant to investigate.

The mine continues to operate and its effluent is discharged in batches into a small lake that also receives discharge from a sewage treatment plant. Treatment consists of precipitation, primary settling and pH adjustment at the mill, followed by secondary settling in holding tanks. Secondary settling takes place in several 5 000 m^3 outdoor ponds which are discharged periodically or are recycled, depending on the results from the pond filling sample. Pond samples are analysed frequently to ensure optimum effluent quality prior to release. The effluent essentially consists of three process streams: two raffinate streams and minewater. The term raffinate refers to the aqueous wastewater from a solvent extraction unit. There are two such circuits, the main uranium purification circuit which uses a mixture of kerosene, isodecanol and a tertiary amine, and a molybdenum removal circuit which uses a LIX extractant (where LIX is the commercial name for the reagent). The three effluent sources are combined and treated prior to discharge.

Mill personnel suspected that loss of solvent in raffinate from one of the solvent extraction circuits was the cause of toxicity. The suspected increased solvent losses correlated with a change in ore characteristics, which had manifested itself in other ways in the mill circuit (generation of hydrogen gas during leaching). Therefore, it was not possible to automatically assume solvent loss as the cause of toxicity. The potential presence of trace organics in the ore could also not be ruled out. These various concerns, coupled with the possibility of some new additive or synergistic behaviour between various low grade sources of toxicity led to the decision to undertake a systematic investigation into the recently encountered acute toxicity problem found in routine standard protocols.

The TIE study successfully identified the compound responsible for toxicity. The mine conducted an in-house TRE following the TIE study in order to reduce the levels of this compound. As it turned out, the root cause did not turn out to be one of the solvent compounds. The TRE steps involved process modifications, increased treatment efficiency (use of increased aeration and heat), and toxicity monitoring using the Microtox® system. The TRE process is continuing and is directly related to the findings of the TIE study.

The manager responsible for environmental protection at the corporate office replied to the AETE TIE questionnaire. He provided the consultant's TIE report, which was detailed and fairly complete. This particular TIE was thereby selected for a case study. The manager provided further details regarding mill operations and effluent treatment over the telephone. This study is an appropriate selection since it was conducted fairly recently (1993-1994) and was considered successful.

The TIE investigation was conducted over a period of 10 months including initial testing. The causes of toxicity were identified, to the satisfaction of the client, and a TRE program was introduced and monitoring of toxicity for each pond is now routine. According to the mill personnel, the results of the TIE were worth the cost of the study.

3.3.2.1 Overview of the TI/RE Study

The TIE study was conducted by two commercial laboratories - a main contractor who did toxicity studies and a sub-contractor responsible for chemical separation and analyses. The laboratory conducted an initial investigation of historical effluent chemical and toxicity data. The next step was a partial Phase I TIE where the suspected toxicant was partially isolated. This was followed by characterization of the possible chemical nature and structure of the toxicant. Later tests with effluent streams, products in use at the mill, and commercially available products representing classes of compounds confirmed that the isolated toxicant was responsible for toxicity.

The laboratory used a battery test approach - the use of several species of organisms. Toxicity tests involved rainbow trout, fathead minnows, *Daphnia magna* and the Microtox® system. In later toxicity tests, the laboratory was able to demonstrate a relationship between toxicity to trout and the reduction of luminescence in the Microtox® test. This relationship was used by the mill for a continuing on-site monitoring program. Initial toxicity tests showed that the toxicity was persistent. The next step was a partial Phase I TIE. Some protocols were modified to rapid low volume tests used as screening tools (presence/absence of toxicity). Trout were used in whole effluent and confirmation tests.

Preliminary Studies - Initial Testing with Rainbow Trout

The initial testing with rainbow trout is not part of the TIE process, but the consultant used the information gathered at this preliminary stage to design the TIE study and to analyze results. The initial testing was conducted after toxicity was observed in routine toxicity tests with trout. In November 1993, an effluent sample was tested and mortalities were observed in the 100% exposure. A sample collected on January 20, 1994 was also tested. In this case, the sample was split and tested by the consulting laboratory and by the regional Environment Canada laboratory. The full strength sample was again toxic at both laboratories, causing complete mortality within 24 hours.

The consulting laboratory tested the persistence of the sample in a simple yet apparently effective manner. After 24 hours, there was complete mortality in the full strength effluent exposure. The dead fish were removed and replaced with live fish and the test was re-started. All of the replaced fish died within 24-48 hours (e.g., within 48 - 72 hours after the initiation of the original test). The dead fish were again replaced (72 hours after the initiation of the original test) and, again, all of the replacement fish died after 24 hours exposure (96 hours after the initiation of the original test). The persistence of sample toxicity was thus demonstrated in this initial phase of testing. It should be noted that this “fish replacement” technique may be useful to test the persistence of extremely toxic effluents, but may not be effective with moderately or less rapidly acting toxic samples.

The effect of aeration on the reduction of sample toxicity was demonstrated during rainbow trout tests with samples that had low dissolved oxygen levels. The use of aeration permitted by the test protocol evidently reduced the stress of the test organisms.

Survey of Effluent Chemistry

The sample collected on January 20, 1994 was chemically characterized by a contracting laboratory (alkalinity, conductivity, hardness, sodium, potassium, nitrate, nitrites, total Kjehldahl nitrogen (TKN), pH, phosphorous, sulphate, total organic carbon (TOC), metals and ammonia nitrogen). The concentrations of some parameters were compared with historical chemical data collected between February 1992 and October 1993. Values included a geometric mean, maximum and minimum values for some, though not all, of the parameters analyzed in January 1994 (Ca, Mg, ammonia N, phosphorous, K, Na, SO_4, TOC, Al, B, Cd, Cr, Co, Cu, Fe, Pb, Mn, Mg, Mb, Se, V, and Zn).

The consulting toxicity laboratory compared historical effluent toxicity data and related it to chemical concentrations. However, since the chemical data evaluated only covered a period of slightly under two years, at most only five toxicity tests results were available to match with the chemical data (two in 1992, two in 1993 and one in January 1994).

The 1994 data indicated that the effluent was slightly acidic, with elevated levels of hardness, conductivity, SO_4 and ammonia. The consultant eliminated some candidate toxicants based on this review. The effluent had always contained elevated levels of Ca, Mg and SO_4, even in samples which had not caused any toxicity to trout. While levels of total ammonia were elevated, there would be little un-ionized ammonia at the effluent pH of 6. Concentrations of the metals Li, Mb, Ni, Mo and As were also high, but not high enough to cause death to trout within a period of hours (based on information in the literature). In addition to the historical toxicity and chemistry data, conclusions of the possible nature of toxicant were based on results of the battery of tests (to be discussed in the next section).

From the report, the consultant appears not to have evaluated or reviewed the processes and compounds involved in the operation of the mill, its maintenance practices nor those of the treatment facilities. This would have been a logical step since toxicity was observed only recently. With the cooperation of the mill, it should have been possible to identify any novel or recently introduced practices or products that could explain toxicity.

However, further discussions with mill personnel revealed that there was in fact considerable communication between the toxicity laboratory and the mill regarding the toxicity tests and the mill's operations. Information was exchanged which influenced the course of the TIE (and likely increased the efficiency of the process). This was an important factor in the success of the TIE.

Battery Testing

Prior to comparing effluent chemistry data with historical toxicity tests, the consultant conducted a series of test with four test species: rainbow trout, *Daphnia magna*, fathead minnow (*Pimephales promelas*) and the marine bacterium *Vibrio fischerii* used in the Microtox® test system. The battery of tests were conducted on a second sample, collected on January 31, 1994. Some tests with trout and all tests with fathead minnows involved reduced numbers of organisms (one to three per test vessel) reduced test volumes, or single concentration exposures. The modified tests were intended for use as screening tools only (presence/absence of toxicity).

The four toxicity tests were conducted with the January 31 sample. While the full strength sample caused less mortality to trout (10%, compared to 100% for the previous January sample), all of the surviving fish were observed to be evidently stressed (disorientation, change in pigmentation). Fathead minnows exposed to the 100% sample exhibited some mortality (one of three exposed individuals died) and some inhibition of luminescence was indicated in the Microtox®. No toxicity was observed to the cladoceran *Daphnia magna*.

The conclusion was that the fathead minnows and Microtox® were responding to the same toxicant that caused trout mortality. Despite the lack of response of *Daphnia magna*, all organisms were retained for the TIE test battery.

Further information was obtained during the preliminary battery tests by comparing the responses of the different organisms in light of their known susceptibility or tolerance of various toxicants. The consultant compared the responses of trout and Microtox® in the context of the chemical analyses conducted on the January 30th sample. While levels of total ammonia were elevated, there would be little un-ionized ammonia present at the effluent pH of 6. Exposure to the effluent resulted in toxicity in the Microtox® test (52% reduction of light output in the 90% v/v effluent exposure). However, since the marine bacterium used in the Microtox® test was considered as relatively insensitive to ammonia (though no reference was cited to support this contention), the consultant concluded that the toxicity observed in the Microtox® test was not due to ammonia. This provided further evidence to the consultant to eliminate ammonia as a source of toxicity in the effluent.

In addition, during these initial tests, samples of the January 31 effluent were aerated. All fish died within 24 h in the 100% v/v exposure, but aeration of the other effluent concentrations appeared to reduce fish stress and all survived. An assay conducted with Microtox® bacterium six months later (in July) confirmed that aeration reduced toxicity.

Modified Phase I TIE Study

The consultant then proceeded with a modified Phase I study to characterize the toxicant. The consultant considered the review of historical chemical and toxicity data, described previously, as the Characterization step. The next step, Identification, involved fractionation of the sample.

As previously discussed, the consultant eliminated ammonia, sulphate and metals as sources of toxicity. The reasoning is summarized as follows: (i) the toxicant was rapidly acting, so metals, including Ca and Mg, were not suspected; (ii) the effluent pH indicated that there would be little un-ionized ammonia present; (iii) historical effluent samples had contained elevated levels of sulphate and other constituents, yet the same caused little or no toxicity to trout; (iv) results of an aeration treatment in initial testing indicated that the suspect toxicant was either volatile or could be easily oxidized.

Based on this reasoning, the approach selected involved filtration, air sparging (aeration), pH adjustment (acidic, alkaline, ambient), Solid Phase Extraction (SPE) using a C18 column, and SPE followed by elution with methanol. In all, twenty-one treatments were conducted, with toxicity tests involving fathead minnows, *Daphnia magna* and Microtox®. Confirmatory tests were conducted with trout. The treatments were conducted with the January 31 sample.

The report provided by the mining company includes complete test reports for the initial tests and for follow up testing conducted after the TIE. However the TIE test reports for fathead minnow, *Daphnia magna* and Microtox® assays are not as complete as those provided for the initial tests (levels of some physical-chemical measurements such as dissolved oxygen are not included with the TIE reports).

The use of several test species was not entirely successful. Since none of the daphnids died during the TIE, their inclusion added nothing to the interpretation. Problems were also encountered with anomalous mortalities in some of the modified fathead minnow exposures.

Initial effluent tests showed that the exposures did not cause toxicity to *Daphnia magna*, yet the invertebrate was included in the TIE process since it was believed that it may show sensitivity to some methanol elutriates. However, few daphnids died during the TIE testing and these responses were not mentioned in the report, suggesting that they contributed little or nothing to the interpretation.

The fathead minnow test involved three fish in a reduced volume. No daphnids died during the 100% v/v baseline test, while one of the three minnows died. However, in the controls of this test, two of three fatheads died in one of the replicates (for a control mortality of about 22%).

Some of the daphnids in the C18 exposures (25% methanol elution) died, yet the mortality did not appear to be related to the particular effluent treatment. These results suggest caution should be used when using modified protocols. Tests involving reduced volumes and fewer organisms should not be undertaken without more extensive testing and trials, even if they are used merely as screening tools. In addition, no physicochemical data, aside from measurements of conductivity and pH and observations about odour, were provided with TIE reports to determine the cause of these anomalous responses (inadequate oxygen for example).

The results of the toxicity tests involving fathead minnows, *Daphnia magna* and Microtox® are summarized in Tables 3-13 (Phase I), 3-14 (Phase II) and 3-15 (Phase III).

Conclusions of Phase I

The consultant concluded that the cause of toxicity was not due to inorganic compounds or high levels of dissolved salts. Based on initial trout tests and results of the Phase I study, the toxic constituents were volatile or easily oxidizable non-polar organics isolated in the 75% methanol fraction of the SPE step. It was also concluded that the bacterial luminescence test was more sensitive than the other tests.

The mill manager also commented that other lessons were learned. Rapid transport of the sample to the laboratory was important since sample toxicity decreased over time. In addition, elevated sample temperature reduced the toxicity. From the mill personnel perspective, it indicated that the toxicant was possibly coming from one of the raffinate streams, rather than from the mine water. This set the stage for Phase II, with assessment of the various components of the raffinate streams.

Phase II: Identification of the Toxic Constituents in the Effluent

The next process was to identify the substance using gas or liquid chromatography / mass spectrometry (GC/MS, LC/MS). The first step involves separation of the organic compounds (individually or as a class in peaks). The second allows more precise identification and quantification of the relative abundance. Chemical analyses were also performed on two fresh effluent samples, collected on July 12 and 16, 1994. Trout toxicity tests were conducted with the latter samples to confirm toxicity.

The first analysis was for volatile organic compounds on the January 31 untreated effluent sample and in the 75% methanol fraction eluted from the SPE column. This step was not successful, since no compounds were identified compared to a laboratory dilution water control. The most probable case was an inadequate sensitivity (detection of the method was low ppm, too low for many organics).

A more sensitive LC/MS analysis was conducted with the 75% methanol fraction. Two peaks were detected in this sample which were not present in the control dilution water. The molecular weight of the compounds was in the range of 160-200 (daltons).

The July effluent samples were also analysed by GC/MS and LC/MS. Toxicity tests accompanying the sample were done. Both aerated and non-aerated samples were passed through an SPE column at ambient pH, followed by elution with 25% - 100% methanol. Screening tests were conducted with fathead minnows, *Daphnia magna* and Microtox®, and the results were later confirmed with trout. All of the toxicity tests indicated that the 75% fraction was the most toxic (Table 3-14). The chemical analysis indicated that one of the peaks was reduced in the aerated sample, indicating that this peak contained the toxicant, with a molecular weight of about 200 amu. The compound(s) were identified as straight chain aliphatic alcohols or diols, approximately 10 to 12 C atoms long, with possible branching chains. Despite the existence of a vast number of compounds matching this structure, two aliphatic alcohols closely matched the mass spectra - tridecanol and 1,2 dodecandiol.

The aliphatic alcohol is used as a process additive in the solvent extraction and removal circuits. Further chemical treatment of the suspected compound (methylation, acetylation) and analysis supported the conclusion of an aliphatic alcohol.

The results/conclusions of Phase II were that (i) two aliphatic alcohol compounds were identified from LC/MS that corresponded to an aliphatic alcohol used as a process additive; (ii) the molecular weight and amount of these two compounds were determined; and (iii) the concentration of one of the compounds decreased after aeration.

Phase III: Confirmation/TRE

Various process additives were tested in the confirmation step, involving the two waste solvent extraction streams and organic reagents used in the circuit (kerosene, isodecanol, LIX 63-70, and alanine). A commercial solution of decanol was also tested.

Testing was conducted with both non-aerated and aerated samples, using fathead minnows and Microtox®. Confirmatory testing was done with trout. The toxicity of solvent extraction waste streams was reduced after aeration (96 h at 7.5 mL/ min). Analysis of process stream samples (mine water and the two raffinate streams) revealed the presence of isodecanol, as well as similar compounds. The 75% methanol fraction had an identifiable odour, which was also present in the three process streams and the isodecanol solution, but was not present in the kerosene and alanine solutions. These latter compounds were eliminated as possible major toxicants.

Finally, solutions of two related aliphatic compounds, n-decanol and isodecanol were tested with fathead minnows and Microtox®. Pre-aeration reduced the toxicity of n-decanol. Aeration reduced the peak height of the isodecanol solution. Chromatographic peaks of the isodecanol solution matched those from the 75% methanol fraction eluted from the C18 column. This confirmed that the loss of toxicity from aeration was a result of the disappearance of long chain alcohols.

3.3.2.2 Comments on the TI/RE Approach

In general, this was an extremely successful TIE, which was efficiently managed and effective. The consultant identified the specific toxicant and provided the mill with a reliable monitoring toxicity tool (Microtox®) to predict trout toxicity. A key component of this success was communication with mill personnel and the involvement of a knowledgeable organic chemistry laboratory in the identification process.

i) Approach to Phase I TIE

The consultant followed a modified approach to a Phase I TIE. The approach to the Phase I TIE, in particular the choice of SPE, was generally logical and was fairly well explained in the report.

With some exceptions, the choice of treatments is logical in view of the characteristics of the suspected toxicant. Aeration was chosen to strip a volatile compound, reducing toxicity. The pH adjustment (followed by testing at original pH) is logical since changes in pH can have profound effects on a number of chemical and physical properties of toxicants including solubility, polarity, speciation and stability. A change in pH can change the ratio of ionized to un-ionized forms of toxicants with a resultant change in toxicity. A step of pH adjustment can increase the proportions of volatile or oxidizable compounds; if this is followed by aeration, toxicity due to this type of compound should be reduced.

Finally, pH adjustment and use of a C18 column pH adjustment and use of a C18 column pH adjustment and use of a C18 column pH adjustment and use of a C18 column pH adjustment and use of a C18 column pH adjustment and use of a C18 column evaluates the extent to which effluent toxicity may be due to relatively non-polar organics. A sub-sample of effluent at neutral pH is passed through a prepared C18 cartridge and non-polar contaminants are adsorbed onto the C18 material. Prior adjustment of pH will cause any organic acids and bases that are present to become less polar by shifting the chemical equilibrium to the un-ionized forms.

However, it is not entirely clear why the pH adjustment and filtration tests were included. Their intent is to evaluate the effect of pH change and filtration on the toxicity of the effluent by removing particulate matter and precipitates (which may result from pH adjustment). The report presented no evidence that particles or precipitates were suspected of causing toxicity and no explanation is given in the report as to why these treatments were included. One explanation may be the formation of precipitates in the effluent. The manager's comments on a previous version of this case study included the fact that gypsum ($CaSO_4$) in the effluent can form a precipitate. Although not stated, filtration is a necessary pre-treatment of a sample prior to passage through a C18 column. The consultant may have wished to evaluate the effects of filtration and pH adjustment separately from SPE treatment.

Since ammonia did not appear to be implicated in toxicity, dropping the zeolite treatment was logical. In addition, since metals were not implicated, the EDTA chelation or cation exchange column treatments would contribute little additional information. There is no discussion of why the anion exchange treatment was not conducted, especially since only a few anions were measured. This could have been important since any unsuspected anions may have contributed to toxicity. However, the elevated sulphate levels which were not associated with toxicity would have certainly masked any other anions; thereby limiting the usefulness of the anion exchange treatment.

It is also not clear why some other treatments were rejected based on the chemical evidence presented in the report. Many volatile substances are organic compounds, which are not necessarily completely non-polar (e.g., acetic acid). However, treatments involving activated carbon or XAD-4 resin were not considered. Activated carbon and the XAD resin in particular will remove a broad range of organic compounds, including humic substances and organic carbon. One reason for limiting the number of treatments was cost. The manager commented that not all of the treatments were conducted since solvent loss was the suspected cause of toxicity at the beginning of the study.

The use of sodium thiosulfate to reduce levels of chlorine (by oxidation) is well known. However, despite mentioning the possibility that "the toxic compounds were volatile or easily oxidizable", the oxidant treatment with sodium thiosulphate was not attempted.

If the Phase I process is modified, it is possible to overlook classes of compounds that contribute to toxicity. However, a knowledge of effluent chemistry and of the industry can eliminate some Phase I treatments which are unlikely to affect the outcome, reducing costs and effort. In addition, the contact at the mill stated that there was consultation between the laboratory and the mill.

The main comment of this study is that the selection of the SPE for the TIE January 31 sample appears to have been based on only a few pieces of evidence. The evidence for suspecting a volatile organic compound mentioned in the report is one trout test indicating the effect of aeration. However, the mill personnel suspected that the toxicity was due to a solvent and discussed their reasons with laboratory staff.

ii) Approach to Phase III TIE

The identification portion of the study involved an analytical chemistry laboratory. This laboratory conducted an organized progressive search, identifying classes of possible candidate toxicants at an early stage. When one method did not resolve the issue due to inadequate detection limits, an alternative approach was successful in identifying the class of compound responsible. The report provides an impression of skillful analysts involved in this step.

iii) Phase III: Confirmation and Experiments with Components (streams) of the Effluent

The consultant conducted other testing with components of the effluent stream (uranium solvent extraction, mine water and moly removal raffinate) in April and October 1994. In addition, testing of the undiluted effluent ("pass/fail" tests) was conducted between July and December 1994 in conjunction with Microtox® tests, as discussed above. The report focusses most of the discussion on the January and July samples, which underwent the TIE. The other studies provided useful data, yet it was difficult to place these studies in context.

However, the experiments with the effluent streams generally confirmed the presence/use of the suspected toxicant and the contribution of the stream to the overall toxicity of the effluent. The confirmation of the toxicant was conducted using the Microtox®. Some difficulties were observed with the aqueous solubilities of the products tested, yet the overall results confirmed the toxicant's potency.

iv) General Comments

Use of Battery Testing

The use of a battery of tests was less successful than focussing on one or two species (trout and Microtox® for example) would have been. *Daphnia magna* generally experienced no mortalities during the testing, or experienced mortalities where other organisms did not respond. It would have been more efficient to exclude this organism based on results of initial battery tests.

With one sample, there were extensive mortalities (two of three fish in one replicate or, 16%) in the control laboratory water exposures with the modified fathead minnow test. Other tests did not experience these difficulties and the use of the fathead minnows provided useful data. However, the use of reduced test volumes and numbers of animals in modified tests needs to be examined.

Testing of Other Effluent Samples

Several toxicity tests were conducted over the course of the study, yet the report concentrates on discussion of the January samples. The consultant also conducted tests on some components of the effluent (the two raffinate streams) on samples collected later in the year. The characteristics of these particular samples are not compared with the previous ones, though the testing did contribute to identifying the source and nature of the toxicant. It is perhaps helpful for the success of the TIE that the effluent characteristics were stable and did not vary over time.

Lack of statistics

With the exception of LC50s, there is no statistical treatment of the data. In this case, the treatment effects were generally "toxic" or "non-toxic" so this is not a serious deficiency.

However, the consultant undertook Microtox® and trout testing with 18 effluent samples during the period covered by the report. The toxicity data were compared and a guideline was established for using the Microtox® as a monitoring tool to predict trout toxicity. However, no correlation was calculated for the trout and Microtox® data since the data were generated using undiluted samples (100% v/v). The guideline was actually a threshold in the response of the Microtox® below which one would expect trout toxicity to occur. The Microtox® was more sensitive than trout to the toxicant. An effluent that would be toxic to trout usually caused an inhibition of 80% or more, compared to the controls, in the Microtox® test. The mill personnel currently use this relationship to monitor the effluent and the dispersal of toxicity downstream.

In the data set provided, there are only six trout failures out of the 18 tests, of which five entailed 100% mortalities The corresponding Microtox® response for these five tests ranged from 91 to 79% inhibition of bacterial luminescence. However, in one test, the Microtox® response was 80%, while no mortalities were recorded in the trout test. The Microtox®-trout relationship would be more accurate if an actual correlation, with confidence limits, had been calculated. This would require LC50/IC50 data and could be used for the same purposes as data based on the "pass/fail" tests. It is likely that a correlation of toxicity data would provide more certainty for predicting effluent toxicity to trout. However, it should also be stated that such a correlation would be difficult to obtain if the effluent toxicity is not frequent, as appeared to be the case. In addition, the effluent is not presently toxic.

The identification of a rapid effective toxicity monitoring tool, the Microtox®, allowed the mill personnel to purchase their own equipment. As a result, additional TRE assessments could be conducted by the mill personnel. This additional work was not included in the report.

3.3.2.3 Overall Summary of Case Study

- The client was satisfied with the results of the study. It fulfilled regulatory needs to properly identify the cause of effluent toxicity.
- The consultant conducted a review of effluent chemical data and compared effluent parameters.
- A modified Phase I study was conducted focussing on volatile organic contaminants.
- This was followed by identifying the cause of toxicity, an aliphatic alcohol (isodecanol). This lead to process modifications and modifications to the treatment system to solve the toxicity problem.
- The TIE helped to focus on the particular chemical grouping that may have been toxic which agreed with the initial hypothesis that increased solvent loss from the uranium solvent extraction circuit was responsible for the change in effluent toxicity.
- Generally, the TIE study involved a systematic approach to solving the problem.
- Knowledge of the mill processes (changes, upsets, reagent usage) and communication with mill personnel reduce the wasted effort in such studies.
- The report established a basis for using the Microtox® system as a monitoring tool to predict effluent toxicity to trout prior to discharge to the environment. This correlation is used on an ongoing basis to control effluent quality. The effluent is re-treated if effluent toxicity is suspected during process upsets.

Table 3-13. Responses during Phase I TIE involving effluent sampled on January 31, 1994

Treatment Number	Sample Preparation	Test Organism Fathead minnow % Dead	 *Daphnia magna* % Dead	 Microtox® Luminescence % of Control
	Control (laboratory water)	16%		100
	Baseline	33%	0	52
1	Adjust to pH 3 + filtration	33	0	55
2	Adjust to pH 3 + aeration	0	0	136
3	Adjust to pH 3, C18, elution with 25% methanol	0	0	110
4	Adjust to pH 3, C18, elution with 50% methanol	0	0	47
5	Adjust to pH 3, C18, elution with 75% methanol	33	0	14
6	Adjust to pH 3, C18, elution with 100% methanol	0	0	104
7	Adjust to pH 3, C18 column	33	0	169
8	Ambient pH + filtration	33	0	103
9	Ambient pH + aeration	0	0	138
10	Ambient pH, C18, elution with 25% methanol	0	0	122
11	Ambient pH, C18, elution with 50% methanol	0	0	40
12	Ambient pH, C18, elution with 75% methanol	33	0	17
13	Ambient pH, C18, elution with 100% methanol	0	0	134
14	Ambient pH, C18 column	0	0	183
15	Adjust to pH 11 + filtration	0	0	113
16	Adjust to pH 11 + aeration	0	0	146
17	Adjust to pH 11, C18, elution with 25% methanol	0	0	103
18	Adjust to pH 11, C18, elution with 50% methanol	0	0	21
19	Adjust to pH 11, C18, elution with 75% methanol	33	0	101
20	Adjust to pH 11, C18, elution with 100% methanol	0	0	44
21	Adjust to pH 11, C18 column	0	0	184

Table 3-14. Responses during Phase I TIE involving effluent sampled on July 12, 1994

Treatment Number	Sample Preparation	Test Organism		
		Fathead minnow % Dead	*Daphnia magna* % Dead	Microtox® Luminescence
	Control (laboratory water)	0	0	94
12	Ambient pH, C18, elution with 25% methanol	0	33	114
13	Ambient pH, C18, elution with 50% methanol	0	0	29
14	Ambient pH, C18, elution with 75% methanol	100	100	4
15	Ambient pH, C18, elution with 100% methanol	0	0	76
	Ambient pH, C18, elution with 100% methanol	0	100	135
16	Ambient pH, C18 column	100	100	152

Table 3-15. Toxicity testing of process additives: samples tested April and September 1994

Solution	Treatment	Fathead Minnow LC50	Microtox® Luminescence IC50
Raffinate	non-aerated	0.7	1.2
	aerated	1.4	1.7
LIX	non-aerated	4	5
	aerated	7	19
raffinate: LIX mixture (1:1 ratio)	non-aerated	<0.6	3
	aerated	2.2	24

3.3.3 Case Study #3 - TI/RE with Effluent from a Copper/Nickel Mine

The third case study involves a copper-nickel mine. The effluent was consistently toxic to rainbow trout and *Daphnia magna* and the company engaged consultants and researchers from a local university to investigate the cause(s) of toxicity. Following the TIE studies, the mine proceeded with investigations on an effluent treatment option, specifically pH adjustment. The mine reported that the treatability investigations were conducted in response to provincial regulations which set limits on the pH of discharged effluent, rather than a direct result of the TIE process.

The discharge from the mine was described as a sewage works associated with an integrated base metal mining, milling, smelting and refining facility. The plant treats process waters primarily from the 4000 hectare mill tailings area. Yet the total watershed is double this surface area and includes urban runoff, drainage from the mine plant yards and waste piles. Virtually 100% of the process water for milling is recycled water from a location immediately upstream of the treatment plant.

The treatment plant consists of two reactor clarifiers with rake mechanisms. Slaked lime is the primary treatment chemical, but polymer is also added to assist flocculation and settling of metal hydroxides. Sludge from the operation is recycled to the tailings area. To effectively remove nickel from solution, the pH of the raw water must be raised to above pH 10. The pH must then be re-acidified prior to release. At the time of TIE initiation, the pH of the discharged effluent was 10.5. A pH adjustment system was installed and operational in January 1998. Sulphuric acid, manufactured at the smelter site, is used to reduce the pH to a target of 8.8. The effluent had been acutely lethal to trout or *Daphnia magna* in most toxicity tests, but most recently was consistently nonlethal to both species.

The environmental coordinator for the mine responded to the AETE TI/RE questionnaire, was interviewed over the telephone and provided the reports on the copper/nickel mine's TIE and treatability studies. After reviewing the reports, this investigation was considered a suitable candidate for a case study. The study was an appropriate selection since it was conducted fairly recently, included Phase I TIEs as well as effluent treatability studies. The study was selected after the approval of the mine's environmental coordinator, who also provided further information regarding the current status of the effluent and treatment.

A total of 5 studies were initiated between 1993 and 1997 to investigate the cause of effluent toxicity. All testing involved rainbow trout and *Daphnia magna*. TIE studies were conducted by two different consultant/commercial laboratory firms. Additional investigations and effluent treatability studies were planned and carried out by an M.Sc. student and his supervisor at a local University. The Phase I TIE studies both followed the U.S. EPA protocol. The additional toxicity investigations and treatability studies followed a methodology designed by the university researchers (the toxicity tests themselves followed the standardized Environment Canada protocol) as the previous investigations following the U.S. EPA protocols had not provided the type of information required by the mine. The specific studies were as follows:

- 1993 modified Phase I TIE
- 1995 Phase I TIE
- Preliminary pH adjustments study
- Treatability investigations - pH adjustment part 1 - initial investigations
- Treatability investigations - pH adjustment part 2 - confirmation and implementation

The primary cause of toxicity was identified. Secondary causes of toxicity were not identified, but with the recommended pH adjustment, pH levels typically associated with secondary toxicants (near neutral) were not expected to occur during future testing. In the opinion of the mine s environmental coordinator, the results of the TIE were not worth the total cost of the studies since the conclusions were based mostly on speculation, rather than on statistically relevant results. The treatability studies provided more relevant and applicable information particularly related to pH adjustment targets.

3.3.3.1 Overview of the 1993 Modified Phase I TIE Study

The consultant did not include any information or review of historical chemical or toxicity data. Similarly, there was no evaluation of operational/maintenance practices and treatment facilities. Mine personnel noted that it was decided to focus on end-of-pipe chemical characteristics rather than conduct a detailed chemical usage review due to the large number of unknown inputs to the treatment system. A clear statement of the study objective (e.g., identify the cause of effluent toxicity using the U.S. EPA process) was provided along with the dates of sample collection and test initiation (June - August 1993).

The consultant conducted a modified Phase I TIE study to characterize the toxicant(s) in two separate effluent samples. According to the consultant, the modified TIE was conducted since a review of the available data indicated that ammonia and metals were the most likely cause of effluent toxicity. Data used to make this conclusion were not provided in the report and could not be evaluated or verified. The Phase I TIE included graduated pH testing at 7, 8 and 9; filtration at pH 3, 7 and 11; treatment with EDTA. Initial untreated tests were conducted at the pH of the effluent as received (pH 10.2-10.5). The treated samples were adjusted to pH 7 after treatment and not to the initial pH of the effluent (10.2-10.5). The consultants indicated the rationale for selecting pH 7 was based on the concern that pH of the effluent was high enough to contribute to the toxicity of the sample. It was noted that the authors of the U.S. EPA TIE document agreed with their rationale and approach. Baseline tests were conducted so that responses observed in the effluent treatments (e.g., following the manipulations) were compared to the toxicity of an untreated sample.

Rainbow trout and *Daphnia magna* were the selected test species. The consultant noted that a surrogate test organism (e.g., fathead minnows) was not used due to difficulties that could be encountered when trying to relate test results back to the regulatory species. Two types of toxicity tests were conducted; LC50 tests and screening tests. The LC50 tests were conducted according to the Environment Canada test protocols. The effluent concentrations for LC50s were prepared using laboratory water as dilution water. Methods used for screening tests involved exposing 6 fish to reduced exposure volumes of 3 L. Detailed bioassay conditions for the screening test were provided in the methods section of the report.

Chemical analysis of the untreated and treated samples included an ICP 29 element scan and ammonia. Total ammonia in the first sample was approximately 3.6 mg/L, whereas total ammonia in the second sample was approximately 4.2 mg/L. Results from the metals analysis were provided in a table, but not discussed in the text of the report. A summary of toxicity test results for rainbow trout and *Daphnia magna* are provided in Table 3-16. For the first sample collected on July 21, 1993, trout and daphnid mortality was similar in the untreated initial (pH = 10.2) and unadjusted baseline (pH = 10.2) tests (e.g., LC50 = 71%). The second sample collected on August 31, 1993 appeared to be more toxic than the first sample to both trout (LC50=35%) and *Daphnia magna* (LC50=25%). The August 31 unadjusted baseline test (pH 10.5) was less toxic than the initial test (pH 10.5). For both samples, adjustment of the untreated baseline tests to pH 7 eliminated trout toxicity and reduced toxicity to *Daphnia magna*. Adjustment of effluent to pH 8 eliminated trout and *Daphnia magna* toxicity. Adjustment of effluent to pH 9 eliminated *Daphnia magna* toxicity, but residual trout toxicity was observed in the second sample. Residual *Daphnia magna* toxicity observed at pH 7 was eliminated by treatment with EDTA and filtration at pH 11.

Conclusions of Modified Phase I

The consultant concluded that high pH and ammonia was the main cause of toxicity to rainbow trout and *Daphnia magna* and metals were suggested as a secondary cause, based on the following:

- the effluent was more toxic at high pH than at neutral pH and reduction in pH was accompanied by a decrease in toxicity;
- the pH of the effluent and total ammonia concentration was in the toxic threshold range for trout; and
- reduction of daphnid toxicity with the pH 11 filtration and EDTA tests suggested that residual daphnid toxicity at pH 7 was mostly due to metals, however, the specific metals responsible were not identified.

3.3.3.2 Comments on the 1993 Modified Phase I TIE Study

i) Approach to Phase I TIE

Historical toxicity and chemistry data were not provided in the report, therefore is was not possible to review the data or evaluate if the decision to exclude selected TIE treatments was appropriate. Based on the selection of treatments (e.g. graduated pH, EDTA and filtration), it appears that the consultants were expecting the toxicant to be pH dependent and likely a metal and/or ammonia. However as indicated in case study #1, it is possible to overlook classes of compounds that contribute to toxicity if Phase I is modified. The exclusion of certain treatments does not appear to be logical given that this study appears to have been the first toxicity investigation conducted by the mine. In particular, explanations for the choice of certain treatments and exclusion of others were lacking in the report.

A second critique of this study is the lack of raw data provided with the report. Bioassay test conditions (e.g., pH, dissolved oxygen) were also not provided. Similarly, the report did not include a description of how the LC50s were calculated, nor is there any mention of confidence limits. In cases where raw data was provided for the chemical analysis, no reference was made to the data in the text of the report.

ii) Cause of effluent toxicity

It was concluded that ammonia was the most likely cause of toxicity at high pH based on the fact that toxicity was reduced with lower pH and that the pH of the effluent and total ammonia concentration was in the toxic threshold range for trout. However, the report did not provide measured pH values, un-ionized ammonia concentrations or define toxic thresholds. During this review, un-ionized ammonia concentrations were calculated using the chemical parameters provided in the report. Based on a total ammonia of 3.6 mg/L in the first sample collected on July 21, 1993, a temperature of 15°C and nominal pH values (measured at the start of testing) of 7, 8, 9 and 10.2, the corresponding un-ionized ammonia values were 0.01, 0.1, 0.77 and 2.9 mg/L, respectively. Based on a total ammonia of 4.2 mg/L in the second sample collected on August 31, 1993, a temperature of 15°C and nominal pH values (measured at the start of testing) of 7, 8, 9 and 10.5, the corresponding un-ionized ammonia values were 0.01, 0.1, 0.9 and 3.8 mg/L, respectively. Concentrations of un-ionized ammonia in the pH 7 and 8 adjusted samples were below the toxic threshold of 0.37 to 0.66 mg/L as determined by Thurston *et al.* (1981) for rainbow trout. Elevated concentrations of un-ionized ammonia at pH 9 are the most likely explanation of residual trout toxicity. The increased mortality observed in the second sample may also be related to a higher pH and un-ionized ammonia concentration. However, this information must be interpreted with caution since the consultants did not provide the measured pH values.

Residual toxicity to daphnids at pH 7 was suspected to be due to metals since pH 11 filtration and treatment with EDTA eliminated toxicity. This conclusion (not explained in the report) was based on the fact that, i) EDTA will chelate metals and ii) many metals will form precipitates at elevated pH which can be removed by filtration. A summary of chemical parameters for the untreated and filtered (pH 3 and 11) effluent samples were provided in a table, but not discussed in the text of the report. It is not clear if this chemical information was used in any data interpretations or conclusions. For example, it was interesting to note that the copper concentrations in the untreated effluent (0.06 mg/L) was only marginally reduced following filtration at pH 3 (0.043 mg/L), but was not detected following filtration at pH 11. Similarly, nickel concentrations in the untreated effluent (0.32 mg/L) was only marginally reduced following filtration at pH 3 (0.30 mg/L), but was significantly reduced (0.03 mg/L) following filtration at pH 11. Note that detection limits were not provided in the report. It was also unknown if the values provided were for total or dissolved metal concentrations.

Table 3-16. Responses of rainbow trout and *Daphnia magna* during modified Phase I TIE involving effluent from a copper/nickel mine

Treatment	Rainbow trout		*Daphnia magna*	
	Date Sample Collected			
	July 21, 1993	August 31, 1993	July 21, 1993	August 31, 1993
initial tests (pH 10.2-10.5)	LC50 = 71%	LC50 = 35%	LC50 = 71%	LC50 = 25%
baseline tests (pH 10.2-10.5)	LC50 = 71%	LC50 = 71%	LC50 = 71%	LC50 = 71%
baseline tests (pH 7)	nonlethal	nonlethal	EC50 = 80%	LC50>100%
graduated pH (pH 7)	nonlethal	nonlethal	EC50 = 80 %	LC50>100%
graduated pH (pH 8)	nonlethal	nonlethal	nonlethal	nonlethal
graduated pH (pH 9)	nonlethal	LC50 = 71%	nonlethal	nonlethal
filtration at pH 3	nonlethal	nonlethal	LC50>100%	LC50=71%
filtration at pH 7	nonlethal	nonlethal	LC50>100%	nonlethal
filtration at pH 11	nonlethal	nonlethal	nonlethal	nonlethal
EDTA	nonlethal	nonlethal	nonlethal	nonlethal
Chemical analyses	yes	Yes	yes	yes

3.3.3.3 Overview of the 1995 Phase I TIE Study

The consultant did not include any information or review of historical chemical data and effluent toxicity data. Similarly, there was no evaluation of operational/maintenance practices and treatment facilities. A clear statement of the study objective (e.g., identify the cause of effluent toxicity using the U.S. EPA process) was provided along with the dates of sample collection and test initiation (December 14, 1995).

Phase I TIE treatments followed the U.S. EPA guidance documents and were listed in the methodology section of the report. Treatments included: pH adjustments to 3 and 7, filtration at pH 3, 7 and i (the initial pH of the effluent), aeration at pH 3, 7 and i, C18 at pH 3, 7 and 9, oxidant reduction with sodium thiosulfate, treatment with EDTA and graduated pH tests. Because the initial pH of the effluent sample was 10.4, values chosen for the pH adjustments tests were 3 and 7. Initial untreated effluent tests and manipulations were conducted on the day the sample was received. Tests with manipulated effluent were set the next day. Baseline tests were conducted so that responses observed in the effluent treatments (e.g., following the manipulations) were compared to the toxicity of an untreated sample.

Rainbow trout LC50 tests were conducted on all untreated and treated effluent samples. The effluent concentrations for LC50s were prepared using laboratory water as dilution water. The consultant did not indicate why tests with *Daphnia magna* were not included and information on historical effluent toxicity to *Daphnia magna* was not provided. This information may have been valuable since comparisons of species sensitivity is often used in explaining effluent toxicity (e.g., daphnids are more tolerant to ammonia than are trout). Bioassay conditions were not provided in the methods section and it was initially unclear if the Environment Canada test protocols were followed, or if a modified testing approach was used. A review of raw test data (provided in the report as an appendix) revealed that each exposure concentration consisted of 4 fish exposed to 4 L of either treated or untreated effluent.

Chemical analysis of the untreated sample was limited to pH, temperature, conductivity, dissolved oxygen, alkalinity, hardness, ammonia and chlorine. A summary of toxicity test results for rainbow trout are provided in Table 3-17. The initial untreated effluent sample LC50 was 70.7% and complete mortality in the full strength effluent occurred within 4 hours of exposure. It was noted in the report that the results were similar to the % mortality observed in the November regulatory sample (e.g., 90% mortality after 24 hours), but different from the December regulatory sample where only 40% mortality was observed. The consultant suspected the differences between the two December samples (e.g., one collected for regulatory testing and one for TIE testing) were related to differences in the test protocol. A sample collection date was not provided for the December regulatory sample and it was unclear if the differences were related to the test methodology or if the samples were simply collected at different times.

Trout mortality was similar in the untreated initial and baseline tests. The pH of the 100% untreated effluent sample decreased from 10.4 at the start of the initial and baseline tests to 7.8 at completion of the initial test and to 9.7 at completion of the baseline test. Reasons for the decrease in pH and the differences as measured at the end of the initial and baseline tests were not provided, but may have been related to differences in aeration rates (e.g., as noted later during the treatability studies). It was also unclear from the raw data if pH was measured at test completion (e.g. 96 hours) or when complete trout mortality occurred (e.g. after 4 hours of exposure).

Graduated pH testing clearly demonstrated that toxicity was associated with pH; the effluent sample was toxic at pH 10.4, the initial pH of the effluent, but not at pH 7, 8 or 9. A review of raw test data revealed that pH decreased to 7.5 by test completion in both the pH 8 and 9 tests. Filtration and aeration at pH 3 had no effect on toxicity. Filtration at pH$_i$ decreased toxicity (LC50>100%; 1 out of 4 fish died). However, it was noted that the pH of the sample decreased to 9.5 immediately after filtration and was not readjusted to pH$_i$ prior to test initiation. The laboratory noted that a dark yellow substance was retained on the filter paper. A yellow precipitate was also observed in the pH$_i$ aerated sample. The supernatant was tested and no reduction in mortality was observed, however, toxicity was delayed. As observed in the pH$_i$ filtered sample, pH decreased to 9.6 immediately after aeration and was not readjusted to pH$_i$ prior to test initiation. It should be noted that handling during filtration may have also added to the degree of sample aeration, and hence pH decline. Treatment with sodium thiosulfate and EDTA had no effect on toxicity.

C18 treatment at pH 3 resulted in an increase in mortality, while C18 treatment at pH 7 and 9 had no effect on toxicity. The laboratory attributed the pH 3 toxicity to the degradation of effluent components to sulphide precursors resulting from the removal of a binding compound by the C18 sorbent. It is not clear what was meant by this statement. A strong sulphide odour was noted in the pH 3 adjusted samples. However, analysis for sulphide was not conducted and this source of toxicity is speculative. Another possibility not mentioned in the report is that the capacity of the C18 sorbent may have been exceeded at pH 3 resulting in "break through" toxicity. It does not appear that laboratory checked for "break through" toxicity by collecting and testing a separate aliquot of post treated effluent (e.g., 100 mL of C18 treated sample is collected and tested after 50 mL and 200 mL of effluent are treated).

Conclusions of Phase I

The consultant concluded that ammonia was the prime substance responsible for the toxicity to rainbow trout based on the following:

- toxicity was reduced with lower pH and that the pH of the effluent and total ammonia concentration was in the toxic threshold range for trout.

It was also concluded that:

- pH adjustment was the main factor in altering the effluent toxicity to rainbow trout;
- sulphides were the cause of increased toxicity in the pH 3 C18 treated sample; and
- cationic metals were not a likely source of toxicity, but could not be ruled out as a possible secondary source of toxicity.

3.3.3.4 Comments on the 1995 TIE Study

i) Approach to Phase I TIE

The laboratory provided a very thorough summary of initial, baseline and treatment LC50s with 95% confidence limits. Raw toxicity test data were provided in an appendix. Useful information was provided on time to mortality for the initial and baseline tests. It was also noted in the report that complete mortality was delayed, but not eliminated in the pH_i aeration test. However, it is not known if mortality was delayed in any of the other treatments. It would have been useful for the consultant to have provided results from more frequent monitoring events (e.g., 1, 2, 4, 8 and 12 hour mortality results) since valuable information on the characteristics of the suspected toxicants (e.g., persistency) can often be obtained from those treatments which reduce or delay mortality.

A summary of daily effluent parameters (e.g. copper, nickel, pH etc.) monitored by the mine was provided for November discharges in an appendix, but this information was not referred to in the text of the report. It is not clear if this chemical information was used in any data interpretations or conclusions. Chemical data (other than general water quality parameters mentioned previously) were not provided for the December sample under investigation. However, it is possible that the chemical data results were not available at the time of report preparation.

The consultant closely followed all of the Phase I TIE treatments outlined by the U.S. EPA using rainbow trout as the test species. However, there is a common misconception that each Phase of a TIE must be sequential when in fact, they are often completed simultaneously. Based on the total ammonia concentrations and pH of the effluent, the inclusion of zeolite or air-stripping to remove ammonia (listed as Phase II treatments) would have been logical and provided further evidence that ammonia was the main cause of toxicity.

ii) Effluent pH

As observed in this case study, changes in effluent chemistry during toxicity testing can greatly complicate the identification of the suspected toxicant(s). The data suggests that more than one substance is responsible for toxicity and that toxicity appeared to be dependent on pH. Ammonia was suspected as the source of toxicity at high pH, while metals were the likely source at low pH. This type of effect is referred to as a "matrix effect", in that toxicants interact with other effluent constituents in ways that change their toxicity. Identification of pH dependent matrix effects are often not known until the TIE study is initiated.

It was concluded that ammonia was the most likely cause of toxicity based on the fact that toxicity was reduced with lower pH and that the pH of the effluent and total ammonia concentration was in the toxic threshold range for trout. However, the report did not provide un-ionized ammonia concentrations or define toxic thresholds. During this review, un-ionized ammonia concentrations were calculated using the chemical parameters provided in the report.

Based on a total ammonia of 6.2 mg/L, a temperature of 15°C and nominal pH values (measured at the start of testing) of 10.7, 10, 9, 8 and 7, the corresponding un-ionized ammonia values were 5.4, 4.5, 1.3, 0.17 and 0.02 mg/L, respectively. Concentrations of un-ionized ammonia in the pH 7 and 8 adjusted samples were well below the toxic threshold of 0.37 to 0.66 mg/L as determined by Thurston *et al.* (1981) for rainbow trout. Based on this information it was surprising that trout toxicity was not observed in the pH 9 adjusted sample. However, as noted earlier, pH decreased to 7.5 at test completion in both the graduated pH 8 and 9 tests. Unfortunately, it is unknown how quickly pH decreased in these tests since measurements were only conducted at the start (0 hours) and end (96 hours) of each test. The effect of these decreases during pH 8 and 9 testing were not discussed in the report, but are the most likely explanation as to why the pH 9 adjusted sample was nonlethal. The un-ionized ammonia concentrations at pH 7.5 would have been 0.05 mg/L, well below the acutely lethal levels.

Based on the raw data, it is evident that pH was not adjusted or maintained during graduated pH testing. Consequently, the toxicity of the effluent at a constant pH of 8 or 9 is not known. It would have been useful in the interpretation of test results to have known if the effluent was nonlethal at a pH of 8 or 9 and then to have compared the concentrations of un-ionized ammonia at these different pHs. This information would have provided further evidence to support the hypothesis that ammonia was the main cause of toxicity. Filtration was reported to have reduced toxicity (LC50>100%; 1 out of 4 fish died), however, the pH of filtrate at test initiation was 9.5 and decreased to 7.7 at test completion. It is unknown if filtration actually reduced toxicity since the pH of the treated effluent was not readjusted to pH_i (10.5) at the start of testing.

The consultants noted that cationic metals were not a likely source of trout mortality, but the presence of metals or halide toxicity may have been masked by high pH alone or by the toxicant present at high pH. For example, treatment EDTA and sodium thiosulfate had no effect on toxicity, however, the pH at the start and end of testing was 10.2 and 9.8, respectively. The presence of lethal levels of un-ionized ammonia at these pHs would have masked the presence of any positive effect the addition of EDTA or thiosulfate may have had on reducing effluent toxicity. One possible testing option would have been to remove ammonia from the effluent using zeolite or air-stripping at pH 11 and then tested alone and with EDTA or thiosulfate. It is important to note that elimination of toxicity would not imply that ammonia was the only cause of trout mortality, since zeolite can remove cations from solution and air-stripping can remove other substances that are volatile at pH 11. Treatment with cation/anion exchange resins may have been useful in determining if secondary toxicants were present. The laboratory also did not report that the pH of the untreated sample (10.4) was likely high enough on it's own (even without the presence of any other toxicant) to contribute to trout mortality.

iii) Reduced exposure volumes

It was evident from raw data that reduced exposure volumes (2 L) and a reduced number of fish (4 per exposure concentration) were used for testing. As indicated in case study #1, the use of rainbow trout in a Phase I study often requires greater effort and expense since trout require large test volumes. Other TIE studies (e.g. case study #1) avoided this by using a surrogate test species (usually fathead minnows) in place of trout. As in the previous study, the mine avoided the use of a surrogate test species and having to ship large quantities of effluent by the use of reduced exposure volumes.

Given that the bioassays did not follow the standardized Environment Canada test protocol, detailed information as to the testing methodology should have been provided by the consultant. For example, it was unclear if the exposure solutions were aerated during testing and in our experience it is more difficult to control and maintain comparable aeration rates in small volumes (e.g. <5 L). Also of particular concern is the difference in % mortality noted for the December regulatory sample (40% mortality) and the untreated TIE sample (100% mortality). Results from the TIE may be questionable if the samples were collected at the same time and the difference in bioassay methodology was in fact responsible for the difference in test results.

The approach of reduced exposure volumes for TIEs using rainbow trout is currently being investigated by the Pulp and Paper Research Institute of Canada (PAPRICAN). The pulp and paper industry was interested in the development and validation of TIE methodologies using rainbow trout in reduced exposure volumes. The methodology developed involves exposing 3 fish in 2 L of effluent (including 2-3 replicates). In approximately 98% of the studies, the reduced volume test produced LC50s similar to the Environment Canada test protocol. PAPRICAN has also developed an alternative approach to the U.S. EPA protocol which focuses on toxicants common in the pulp and paper industry. Using the reduced volume methodology for trout and the alternative treatment approach, researchers at PAPRICAN were able to identify the substance(s) responsible for toxicity in 24 out of 32 effluent samples (pers. comm. T. Kovacs 1998).

Table 3-17. Responses of rainbow trout during Phase I TIE involving effluent from a copper/nickel mine

Treatment	LC50 (%, v/v)
initial tests (pH_i = 10.4)	70.7
baseline tests	70.7
graduated pH (pH 7)	nonlethal
graduated pH (pH 8)	nonlethal
graduated pH (pH 9)	nonlethal
filtration at pH 3	70.7
filtration at pH 7	nonlethal
filtration at pH_i (10.4)	>100
C18 pH 3	59.5
C18 pH_i (10.4)	70.7
C18 pH 7	nonlethal
aeration at pH 3	70.7
aeration at pH 7	nonlethal
aeration at pH_i (10.4)	70.7
EDTA	no effect
Sodium thiosulfate	no effect
Chemical analyses	yes

3.3.3.5 Overview of the Preliminary pH Adjustment Study

This project was assigned to two summer students hired by the mine in 1995 and was not considered to be a definitive study. However, a limited review was conducted since the mine felt that the data provided useful background information on pH adjustment and changes in effluent chemistry. A clear statement of the study objective was provided in the report along with the dates of sample collection and test initiation (January 1995). The main objective of this study was to conduct pH adjustments of effluent using CO_2 and H_2SO_4 to; i) determine the effect of pH adjustment on effluent toxicity, ii) determine if the use of CO_2 vs. H_2SO_4 would affect effluent toxicity, and iii) identify possible toxic factors or trends based on water chemistry.

One effluent sample was collected and tested each month for 7 months beginning in January 1995.

The treatments included initial tests, pH adjustment to 7 using CO_2 vs. H_2SO_4 and pH adjustment to 9 using CO_2 vs. H_2SO_4. Single concentration tests with rainbow trout and *Daphnia magna* were conducted on all untreated and treated effluent samples. All effluent manipulations and toxicity tests were conducted by a commercial toxicology laboratory.

Chemical analysis consisting of ammonia, pH, conductivity and 29 element ICP scan, was conducted on only 2 (April and May) untreated and pH adjusted samples. The report provided a summary of % mortality for both trout and *Daphnia magna*. Mortality in all untreated trout tests was greater than 50%. Complete mortality was observed in 4 of the 7 untreated tests with *D. magna*, partial mortality (17%) in one sample and 0% mortality in 2 samples.

The report attempted to explain toxicity based on chemical analysis for the samples collected in April and May. Complete trout and daphnid mortality was observed in the untreated May sample. Toxicity was eliminated after pH adjustment to 7 and 9 with both CO_2 and H_2SO_4. Lethal levels of un-ionized ammonia (4.8 mg/L) were observed in the May sample prior to pH adjustment, but levels decreased to <0.06 mg/L after pH adjustment (to pH 7 and 9).

For most samples, pH adjustment reduced or eliminated trout and daphnid toxicity. However, adjustment of the April sample to pH 7 with CO_2 (but not with H_2SO_4), actually increased daphnid toxicity (17% mortality in the untreated sample and 40% mortality in the pH 7 CO_2 adjusted sample). Adjustment to pH 9 with this same sample had no effect on toxicity. Complete trout mortality was observed in both untreated samples, however, toxicity was eliminated in the May pH adjusted samples, but only reduced by 40% in the April sample. It was noted that Ca and Mg concentrations were lower in the April sample compared to the May sample in which toxicity was completely eliminated. The students hypothesized that the lower hardness effluent (April sample) may have increased metals toxicity. It may also be possible that the large amount of CO_2 added during pH adjustment to 7.0 may have produced CO_2 toxicity. Low dissolved oxygen was also noted in April sample (5.3 mg/L) and was thought to have contributed to trout mortality.

Conclusions from the Preliminary pH Adjustment Study

Based on the above test results, the report concluded that:

- pH and high ammonia were the likely cause of toxicity in selected samples
- metals (Cu and Ni) were also a possible source of toxicity at reduced pH
- pH adjustment to 7 using H_2SO_4 was the most successful in reducing toxicity to both species

3.3.3.6 Comments on the Preliminary pH Adjustment Study

Although it was clear that pH adjustment reduced and in some cases eliminated toxicity, the limited chemical analysis (2 samples only for metals and ammonia) made it difficult to draw any meaningful conclusions as to the cause of toxicity. Furthermore, treatments outlined in the methods section were not repeated consistently with all samples and no explanation was provided. Lack of treatment consistency resulted in difficulties in drawing meaningful conclusions as to which method of pH adjustment was best (e.g. CO_2 or H_2SO_4). Information obtained from mine personnel indicated that the tests were not replicated due to cost considerations and the differences in mortalities could have been related to natural experimental error.

Mine personnel felt that the major conclusion from this study was that chemistry of the effluent changed dramatically from the time of sampling to the time of testing, so relating chemical data at the site to toxicity was expected to be difficult. The conclusion was noted by the mine (but not in the report) when comparing the daily effluent monitoring parameters to results from chemical analysis conducted on effluent collected at test completion.

3.3.3.7 Overview of Treatability Investigations - pH Adjustment Part 1 - Initial Investigation

Treatability investigations were conducted by an M.Sc. student and his thesis supervisor at a local University. This approach was chosen, in part, because two consecutive TIE based studies had failed to provide the kinds of information the mine required for planning practical improvements in it's effluent treatment procedures. The studies were conducted in two parts. Results from part 1, the initial investigations, are discussed below. The report provided a detailed summary of previous work and results generated. A clear statement of the study objective was provided along with the dates of sample collection and test initiation (December 14, 1995). The overall objective of this study was to identify the highest pH (smallest additions of H_2SO_4) that would consistently minimize effluent toxicity. Identifying the specific circumstances leading to toxicity was also given high priority, but the approach used did not follow the U.S. EPA protocols. The researchers stated that a "weight of evidence" approach was going to be used in assessing which substance(s) were responsible for effluent toxicity. The main hypotheses were as follows:

- un-ionized ammonia was the most significant agent of mortality for both test organisms.
- calculation of un-ionized ammonia would be in error if based only on pH levels measured at test initiation rather than during critical times through out the test.
- previous records of unexplained variation in effluent toxicity were often simply a reflection of differences in the extent to which pH declined before or during a toxicity test, and the pH declines were a result of the $Ca(OH)_2$ enriched effluent coming into equilibrium with atmospheric CO_2.
- if thiosulfate and other incompletely oxidized forms of sulfur were present, further acidification would occur at some time following sample aeration via microbially enhanced sulfur oxidation and the generation of H^+ and sulfate ions.

The researchers also recognized the possibility of toxicity due the presence of metals, but thought that this possibility would emerge from the extensive data set.

Five replicate effluent samples were obtained at the beginning of each of 5 weeks during the fall of 1995. Each sample was thoroughly mixed and separated into 15 portions for testing. The report clearly listed each set of experiments as follows (note that as expected, the initial effluent pH was >10):

Experiment A:

- pH adjustments to targets of 9.3, 8.8, 8.3 and 7.8 (one sample was left at the initial pH of the effluent).
- samples were taken for chemical analysis at the mine and then the remaining primary sample was shipped to the toxicology laboratory for testing (post-test sub-samples were also chemically analyzed).

Experiment B:

- 5 sub-samples were vigorously aerated and then allowed to stand for 90 minutes.
- these samples were passed through 0.45 µm filter (with binder) using positive pressure filtration.
- 4 of the 5 filtered samples were pH adjusted to targets of 9.3, 8.8, 8.3 and 7.8 (the fifth sample acted as a control).
- samples were taken for chemical analysis at the mine and then the remaining primary sample was shipped to the toxicology laboratory for testing (also submitted filters for residue analysis and post test sub-samples were also chemically analysed).

Experiment C:

- 4 sub-samples were pH adjusted to targets of 9.3, 8.8, 8.3 and 7.8 (one sample was left at the initial pH of the effluent)
- each sample was then vigorously aerated for 30 minutes.
- samples were taken for chemical analysis at the mine and then the remaining primary sample was shipped to the toxicology laboratory for testing (post-test sub-samples were also chemically analyzed).

Samples for chemical analysis were stored and not analyzed until the toxicity tests were initiated (approximately 24 hours after treatment). The researchers noted that this approach was intended to simulate the period during which the samples were in transit to the toxicology laboratory and thus to maximize chemical equivalency between the effluent being chemically analyzed and the effluent being used for toxicity testing. Sub-samples of effluent were also taken from each exposure at the end of the trout assay for chemical analysis. Chemical analyses included DOC, TOC, total ammonia, COD, suspended solids, sulfate, nitrate, chloride, thiosulphate, conductivity, alkalinity, Ca, Na, Mg, K, Fe, Mn, Al, Ni, Cu, Zn, and Co. Separate samples were filtered and analyzed for dissolved parameters.

All tests were conducted using single concentration rainbow trout and *Daphnia magna* bioassays according to the Environment Canada protocols. At the request of the researchers, each sample was aerated for a minimum of 30 minutes prior to testing, but then followed protocol for pre-aeration with oxygen.

Results from the three experiments indicated that manipulation of effluent samples had little effect on effluent chemistry when compared to the untreated effluent. However, effluent chemical parameters varied considerably among the five different sampling dates. The researchers noted that difficulties were encountered with accurate adjustment to the pre-selected pH values. It was suspected that cold sample temperatures reduced electrode accuracy at the time of acid addition. Measurements of pH recorded after 36 hours of storage were thought to best represent the actual pH adjustments achieved. Comparisons between the effluent stored for chemical analysis and the effluent at the start of toxicity testing revealed that pH dropped rapidly after adjustment.

Fourteen of the fifteen untreated samples were toxic to trout (100% mortality). Only the first untreated sample was toxic to *Daphnia magna* (>50% mortality), but immobile daphnids were observed in many of the untreated samples. Reducing pH clearly mitigated effluent toxicity; high survival was recorded for both species where the pH target was 8.8 or 8.3. Trout survived in all treatments at pH 8.3 and only 10% mortality was observed in two treatments at pH 8.8. The report indicated that *Daphnia magna* mortality was greatly reduced at pH 8.3 and 8.8, however, many organisms (3-47%) were immobile. Trout survival was ≥80% at pH 7.8, but two unfiltered samples at pH 7.8 resulted in 27% daphnid mortality. At pH 9.3, significant trout mortality (60%) and daphnid mortality (27%) occurred in only one sample.

Based on the above data and recognizing that measured pH values would likely be better predictors of mortality than "target" values, the researchers attempted to relate mortality data to measured pH values. The following relationships were examined.

- Relationship between toxicity and pre-test pH:

  Comparisons between mortality and pH of the effluent stored for chemical analysis revealed that for all experiments, mortality rates were lower where pH was between 7.5 and 9.

- Relationship between toxicity and sample pH at critical mortality periods:

  Trout mortalities were typically observed at the end of the 12-24 hour period. Daphnid mortalities were observed in the final 24-48 hours. However, pH was only measured at 0, 24 and 96 hours in the trout assay and at 0 and 48 hours in the daphnid bioassay. It was therefore necessary to approximate the pH levels at the 12-24 hour and 24-48 hour time periods. Not surprisingly, this exercise provided little additional information as to the cause of toxicity, particularly since the test conditions were very different for trout and daphnids (e.g. trout tests are aerated).

Following the above analysis, mortalities observed under alkaline conditions were considered separately from those observed at near neutral pH assuming that the mortalities resulted from at least two different mechanisms of toxicity because un-ionized ammonia could not be present at toxic levels near pH 7.

i) Explanation of toxicity at high pH

Ammonia was considered to be the main cause of toxicity at high pH. Comparisons between toxicity and un-ionized ammonia concentrations (using estimated pH values) indicated ≥ 50% trout mortality was associated with pH values ≥ 8.3 and *Daphnia magna* mortality was ≥ 50% when pH was ≥8.6. In most instances, ≥50% trout mortality occurred when un-ionized ammonia exceeded 0.5 mg/L. *Daphnia magna* were not surprisingly reported to be more tolerant to ammonia. In four samples, ≥50% daphnid mortality was observed when un-ionized ammonia was

≥3 mg/L. However, in one sample 77% daphnid mortality occurred at an un-ionized ammonia concentration of 1.4 mg/L. Similarly, in two samples trout mortality was less than expected given the calculated un-ionized ammonia concentration. Interestingly, the researchers noted that ammonia stripping may have occurred during prolonged test pre-aeration resulting in an underestimation of the concentration of un-ionized ammonia to which fish or daphnid were exposed. Reasons were not provided as to why samples for ammonia analysis were not taken immediately prior to test initiation (e.g., immediately prior to the addition of fish or daphnids to the pre-aerated test solution). However, the researchers did note that comparisons between pre and post chemical parameters suggested that there was little change in total ammonia concentration and, since only unionized ammonia would be lost, the effect on total ammonia concentration would not likely be detectable unless pre-aeration was prolonged.

Because the first effluent sample collected was considered to be the most toxic, the researchers decided to consider an effluent constituent non toxic if it had been recorded at relatively low levels in this sample and at elevated levels in any of the other samples. Using this criteria, comparisons with other measured parameters did not suggest than other substances contributed to toxicity at high pH, but the authors did note that high pH alone could have contributed to toxicity.

The authors presented several theories attempting to explain why either lower or higher than expected mortalities were observed assuming that ammonia was the sole source of toxicity at alkaline pH. While the researchers were very thorough, the explanations were based on only one or two data points and were considered speculative, particularly since the concentrations of chemicals to which the organisms were actually exposed were unknown. The data points in question may not have been "outliers" if the exposure concentrations were actually measured. For these reasons the hypotheses were not presented.

ii) Explanation of daphnid toxicity at near-neutral pH

As noted by the researchers themselves, the explanation for daphnid toxicity was based on a very complex hypothesis. This was also the only section of the report in which statistical analysis was applied. *Daphnia magna* toxicity exceeded 50% in two samples at near neutral pH, a response observed in other studies. Metals were the suspected cause of toxicity, but the specific metal(s) responsible were not identified. The researchers hypothesized that toxicity resulted from some form of precipitate, rather than one of the dissolved metals. This was supported by the fact that the highest daphnid mortality was observed in the sample with the lowest levels of dissolved metals. Analysis of filter residue also indicated that particulates in this sample had a high metals content. It was suggested that Co, Zn and possibly Ni adsorbed to effluent particulates were the agents of toxicity, because filtered effluent was not toxic at near-neutral pH and because filtered residues with high Co/Mn, Zn/Mn and Ni/Mn ratios were positively correlated with samples that caused mortalities. Correlation coefficients were not provided in the report. Mn was considered in the ratios since it appeared to be more important in trace metal complexation. It was suspected that daphnids were more affected than trout because of their closer association with the metal bound particulates (e.g., via filter feeding).

Conclusions on Part I treatability study

The main conclusions drawn from the initial treatability studies were as follows:

- Effluent pH declined rapidly after adjustment and during toxicity testing.
- Considerable effluent variability was observed between samples. Filter residues also varied among samples.
- Pre-test pH was considered to be a useful parameter, but should not be used in interpreting or predicting mortality rates.
- Ammonia was the most likely cause of toxicity at high pH. High rainbow trout mortality was observed at pH levels > 8.5 and un-ionized ammonia concentrations >0.5 mg/L. High *Daphnia magna* mortality was observed at pH levels > 9 and un-ionized ammonia concentrations >2.0 mg/L.

- Metals bound to particulates and consumed by *Daphnia magna* may have been a source of toxicity, however, this hypothesis was based on a single sample and still remains unproven.

Selected recommendations for further investigation included the following;

- Experimental evidence should be obtained to confirm or reject the hypothesis that the first phase of sample pH decline is the result of sample equilibration with atmospheric CO_2.
- Assess to what effect opening sample containers prior to toxicity testing and subsequent sample aeration may decrease alkaline effluent toxicity by increasing the escape of gaseous ammonia.
- Determine the source of pH decline.
- Establish whether *Daphnia magna* may attempt to digest particulates in the effluent being tested.

3.3.3.8 Comments on Part I Treatability Study

i) Approach to treatability study

The overall objective of this study was to investigate the use of pH adjustment to eliminate toxicity and to meet the provincial regulations for effluent pH limits. Previous research indicated that effluent chemistry changed dramatically from the time of sampling to the time of toxicity testing. Consequently, effluent samples that were pH adjusted at the site and then shipped to the laboratory were expected to mimic changes in effluent chemistry that would occur if pH adjustment was selected as a treatment option. Analysis of effluent chemistry immediately after adjustment was also useful since this data would be representative of changes in effluent parameters at the site at the time of discharge.

The main critique of this study was the attempt to explain trout and daphnid toxicity without having measured the concentration of chemicals (particularly pH) to which the organisms were exposed during testing. Although chemical analysis was timed to coincide with initiation of the toxicity tests, the samples were stored at the mine site under conditions that were not representative of the effluent to which the organisms were exposed. For example, at the request of the researchers each sample was aerated for a minimum of 30 minutes prior to testing, but then followed protocol requirements for pre-aeration. The length of pre-aeration varied for each set of experiments which may have resulted in changes in effluent chemistry that would not be accounted for in the samples stored for chemical analysis. The researchers also noted in their conclusions that the intention to study how specific pH levels would influence toxicity was complicated by the fact that pH declined rapidly following toxicity test initiation. However, it is unknown why pH was not monitored more frequently, particularly when the historical data clearly indicated that toxicity was highly dependent on pH.

The filtration and aeration experiments did not follow the U.S. EPA protocol and no explanation was given as to why either treatment was conducted before pH adjustment. By adjusting pH prior to filtration, those compounds typically in solution at ambient pH, but insoluble or associated with particles at more extreme pHs can be removed. Similarly, during aeration some substances can be removed or oxidized most easily at one pH whereas others are most easily removed or oxidized at a different pH (U.S. EPA 1991a). For example, air-stripping at pH 11 is a recognized approach for the removal of ammonia from solution. The efficiency of this procedure at pH 10 is greatly reduced.

ii) Changes in effluent chemistry

In this case study, pH adjustment either reduced or eliminated toxicity in the majority of samples even though the effluent chemistry changed during transport and during testing. To ensure the treatment was consistently effective, a larger number of trials would be required to encompass as many different effluent conditions as possible (e.g. different processes, seasonal variation, flow rates etc.). In particular, further investigations as to the cause of pH decline may be required in order to maximize the efficiency of the treatment and eliminate toxicity at near neutral pH.

Changes in effluent chemistry during transport from the mine site to an analytical or toxicology laboratory is a problem often encountered by isolated industries. Recognizing that it would not always be possible to immediately test a sample for acute toxicity, the Environment Canada test protocol allows for a five day holding period prior to test initiation. It is the sample as received by the testing laboratory on which the mine effluent is judged to be nonlethal or acutely lethal. Similarly, it is under these conditions (e.g., including the delay in transport) that the causes of toxicity are investigated and effluent treatability is assessed. It is recognized that these conditions do not represent the characteristics of the effluent as it discharged, thus chemical analysis must be conducted on the effluent samples to which the organisms are exposed. On-site testing would greatly reduce complicating factors related to changes in effluent chemistry during transport to the testing laboratory, however, this approach is often not economically feasible. Alternatively, the use of rapid screening micro-scale tests (e.g. Microtox®) may be an economical choice if a correlation could be established between the screening test and regulatory test species.

iii) Lack of statistics

The researchers noted in the report that their study generated over 8000 quantitative observations, yet statistical analysis of the data was limited to a few correlations. While individual sample comparisons are often useful in determining treatment effect, large amounts of data can become unmanageable and difficult to interpret without some statistical analysis. Because of the high variation in effluent chemistry, the researchers did not find it appropriate to regard the samples as replicates. While it was agreed that the samples could not be considered replicates, the chemical and mortality variations observed in individual samples may have been useful in providing statistical evidence as to the cause of toxicity. For example, multiple regression models can be used to relate mortality to measured chemical concentrations; however, for this analysis to be successful the effluent must demonstrate a wide range of toxicity with several effluent samples to provide an adequate range of effect concentrations (U.S. EPA 1993b). Although this confirmation method is by no means simplistic, particularly when matrix effects are encountered (e.g., binding of contaminants such that they are made less toxic), the regression analysis may have provided additional evidence to support the suspected causes of daphnid mortality and immobility. Note that this approach would not have been useful with trout, since partial mortalities were only observed in a few samples.

3.3.3.9 Overview of Treatability Investigations - pH Adjustment Part 2 - Confirmation and Implementation

Results from part 2 of the treatability studies are discussed below. As in part 1 of the treatability studies, the researchers were very thorough and provided complete raw data for all toxicity tests and chemical analysis. The report also provided a detailed summary of previous work and results generated. A clear statement of the study objective was provided along with the dates of sample collection and test initiation (April 28, May 5 and May 12, 1997). The overall objective of this study was to confirm that modest pH reductions using sulfuric acid would consistently eliminate the toxicity of the alkaline effluent to rainbow trout and *Daphnia magna.* The main hypothesis was that the primary agent of toxicity was un-ionized ammonia, accompanied by an alkaline pH. It was expected that calculated un-ionized ammonia values would be related to observed mortality rates if pH measurements coincided with critical mortality periods. The researchers also intended to examine the hypothesis that certain trace metals associated with iron-manganese oxyhydroxide precipitates may become labile at near-neutral pH and subsequently toxic to *Daphnia magna* if ingested.

Three effluent samples were obtained at the beginning of each of 3 weeks during the spring of 1997. Each sample was thoroughly mixed and separated into 3 portions for testing (Experiments E, F and G). The report clearly listed each set of experiments as follows:

Experiment E:

- 4 replicate samples each adjusted to pH targets of 9.1, 8.5 and 7.6 (one sample was left at the initial pH of the effluent).
- sub-samples were taken for chemical analysis at the mine and then shipped to the toxicology laboratory for rainbow trout and *Daphnia magna* testing.

Experiment F:

- 4 replicate samples were each adjusted to pH targets of 9.1, 8.5 and 7.6 (one sample was left at the initial pH of the effluent).

- these samples were passed through 0.45 µm filter (with binder) using positive pressure filtration.

- sub-samples were taken for chemical analysis at the mine and then shipped to the toxicology laboratory for *Daphnia magna* testing.

- filters were retained for residue analysis.

pH was carefully monitored before, during and immediately after all treatments. Samples for chemical analysis were taken from the untreated effluent at pH_i immediately prior to manipulation (e.g., pH adjustment and pH adjustment/filtration), stored and not analyzed until the toxicity tests were initiated (approximately 36 hours after treatment). As in part one of this investigation, the researchers noted that this approach was intended to simulate the period during which the samples were in transit to the toxicology laboratory. Sub-samples of effluent were also taken from each exposure at 15 hours into the trout test and at the end of the trout and daphnid tests for chemical analysis. Daphnid specimens from each test were also collected at the end of each test and preserved in 70% ethanol for possible analysis. Chemical analyses included total ammonia, COD, nitrate, chloride, sulfate, thiosulfate, conductivity, alkalinity, Ca, Na, Mg, K, Fe, Mn, Al, Ni, Cu, Zn, and Co. Separate samples were filtered and analyzed for dissolved parameters.

All bioassays were conducted using single concentration rainbow trout and *Daphnia magna* bioassays according to the Environment Canada protocols. At the request of the researchers, all samples for trout testing and daphnid testing were pre-aerated for 90 minutes and 30 minutes, respectively. This variance to the protocols was added to ensure that handling conditions were similar among all samples and replicates. Prior to test initiation, dissolved oxygen, pH, temperature, conductivity and hardness was measured. In the trout tests, pH was monitored at 0, 15, 24, 48, 72 and 96 hours. In the daphnid tests, pH was monitored at 0, 24 and 48 hours.

Results from the two experiments indicated that chemical variability was low among the four replicate samples, but was high among the three different sampling dates. Target pH's were easily than 10 in all samples. For all unadjusted samples, pH declined to approximately 9.6 by the 15th hour of trout testing. For the pH targets of 9.1, 8.7 and 7.6, pH declined to near-neutral by the 15th hour of trout testing. A similar pH decrease was observed during tests with *Daphnia magna*, however the changes were less than those observed with the trout assay (mean decline of 0.4 pH units).

All untreated samples were lethal to trout (100% mortality) and *Daphnia magna* (80% mortality). Samples adjusted to pH 7.6 and 8.7 were nonlethal to trout. Partial trout mortality (2.5%) was observed in one sample adjusted to pH 9.1. All daphnids survived in pH 8.7 adjusted effluent and in pH 8.7 adjusted/filtered samples. Partial daphnid mortalities (2-3%) and immobile organisms (1-19%) were observed in samples adjusted to pH 7.6. Similarly, partial daphnid mortalities (1%) and immobile organisms (2-7%) were observed in samples adjusted to pH 9.1.

The majority of trout mortalities occurred during the initial 15 hours of testing and all mortalities in the unadjusted samples occurred within 2 hours of exposure. All *Daphnia magna* were reported as immobile within 24 hours of exposure to the unadjusted effluent. Mortality could not be confirmed at this time since the organisms must be examined microscopically at test completion (48 hours). Based on this information, the researchers attempted to relate mortality to measured pH values. Complete trout mortality was observed whenever pH exceeded about 10 at 0 hours or 9.5 at 15 hours, while complete survival was observed between pH 9.1 and 8. Crude interpolations suggested that ≥50% trout mortality would occur if pH was ≥9.6 at 0 hours or ≥8.7 at 15 hours. In comparison, *Daphnia magna* mortality was ≥75% when the 0-24 hour pH value was ≥9.3, while almost all daphnids survived when the 0-24 hour pH value was < 9.0. By interpolation, ≥50% daphnid mortality would occur when the 0-24 hour pH was ≥9.2. Average values of the 0 and 24 hour pH measurements were used since, unlike the trout tests, the pH levels at 0 and 24 hours were not clearly different during daphnid testing. A strong relationship was observed between un-ionized ammonia concentrations and trout and daphnid mortality.

Complete trout mortality was observed when un-ionized ammonia concentrations were ≥ 3.3 mg/L at 0 hours or ≥ 2.8 mg/L at 15 hours. Daphnid mortality was ≥75% when un-ionized ammonia was ≥ 3 mg/L.

The researchers suspected that the cause of pH decline was related to the establishment of equilibrium between atmospheric CO_2 and the lime ($Ca(OH)_2$) enriched effluent. The greater decline of pH in the trout tests was thought to be due to differences in aeration protocols (e.g. trout tests were pre-aerated longer than the daphnid tests; trout solutions are aerated during testing, while daphnid test solutions are not aerated) which would have allowed carbon dioxide to be taken up earlier and more rapidly in the trout test.

Recognizing effluent pH would decline during transport to the testing laboratory, it was recommended that in order to consistently pass acute static toxicity tests (e.g., <50% mortality) the effluent pH should be adjusted to within the range of 8.7-8.9. For typical total ammonia concentrations measured at the mine site (5-16 mg/L), pH adjustment to about 8.8 at the site should result in a measured pH of 8.5 at the start of toxicity testing and a reduction of un-ionized ammonia values to less than 2 mg/L.

Conclusions on part 2 treatability study

The main conclusions drawn from the part 2 treatability study were as follows:

- Effluent that was not pH adjusted was acutely lethal to rainbow trout and *Daphnia magna*. Mortality was < 50% in all samples that were pH adjusted to 9.1, 8.7 or 7.6.
- High pH and ammonia were responsible for effluent toxicity.
- Toxicity test results revealed that effluent sample could be rendered nonlethal to rainbow trout and *Daphnia magna* if the effluent was adjusted to approximately 8.8 using sulfuric acid.
- Consistent toxicity results were obtained by ensuring that sample handling conditions were similar before and during toxicity testing.

3.3.3.10 Comments on Part 2 Treatability Study

i) Approach to treatability study

The overall objective of this study was to confirm that modest pH reductions using sulfuric acid would consistently eliminate the toxicity of the alkaline effluent to rainbow trout and *Daphnia magna*. The current study design was based on the initial (part 1) treatability investigations and many of the criticisms of part 1 were addressed by the researchers in part 2. For example, in the current study pH and mortality were monitored more frequently and samples were pH adjusted prior to filtration (rather than after). These important changes to the study design allowed the researchers to provide stronger evidence that ammonia and high pH were the main causes of effluent toxicity.

As in the initial investigations, samples for chemical analysis were stored at the mine site under conditions that were not representative of the effluent to which the organisms were exposed. Although pre-aeration of the test solutions was standardized to reduce between-test variability (unlike the initial study), the sub-samples for chemical analysis were not pre-aerated. It is unknown why sub-samples were not taken from the test solutions following pre-aeration and then preserved for analysis. Samples taken for chemical analysis after pre-aeration would have best represented the concentration of chemicals to which the organisms were exposed since differences in effluent storage and handling conditions would have been minimized.

The researchers noted that when compared to literature values, rainbow trout and *Daphnia magna* appeared to be more tolerant to un-ionized ammonia in the mine effluent. It was suspected that ammonia may have formed complexes with other chemicals in the effluent which could be less toxic than uncomplexed ammonia. However, the interpretation of ammonia toxicity in these effluents was confounded by the fact that pH was continuously decreasing with a subsequent decline in the concentration of un-ionized ammonia. Literature values are likely based on tests were pH remains constant for the duration of the exposure. Another possibility is that the method of analysis may have overestimated the actual concentration of ammonia in the samples.

In the current study, all samples were analyzed using an ion specific electrode which, according to Standard Methods (APHA 1995), is applicable over the range from 0.03 to 1400 mg ammonia / L. However, in our experience with other industrial effluents, we have noted interferences with this method which resulted in an overestimation of ammonia concentrations. Amines and high concentrations of dissolved ions are noted as interference with the ion electrode. It is also noted that for high ammonia concentrations (e.g., >5 mg/L), a distillation and titration technique is the preferred analytical method (APHA 1995). Given that the toxicity thresholds for ammonia were higher than the literature values, it may have been useful to confirm the measured ammonia values using another analytical method (e.g. nesslerization method with distillation).

ii) Lack of statistics

As in the initial investigations, the statistical analysis of the data was limited. It is unknown why the data were not analyzed statistically given the large amount of data generated during both the initial and current study. The researchers noted that none of the other measured parameters (e.g. metals) showed a relationship with trout or daphnid toxicity. The statistical approach used to reach this conclusion was not provided. It appeared that most of the conclusions as to the cause of toxicity were based on graphical comparisons of the data. While this approach was likely adequate when the toxicant concentration versus effect was dramatic and obvious (e.g., ammonia), statistical analysis would have been useful in cases where the cause of toxicity was more subtle (e.g., when trying to explain partial daphnid mortalities/immobilities observed at near-neutral pH).

3.3.3.11 Overall Summary of Case Study

- The client, a copper/nickel mine, did not feel the results from the TIE studies were worth the cost since the conclusions were based mostly on speculation. However, the treatability studies were worth the cost since they provided numerical support and confidence for the conclusions as well as a specific treatment objective.
- A total of 5 studies were initiated between 1994 and 1997 to investigate the cause of effluent toxicity.

- The combined results from all studies suggested trout and daphnid mortality were highly dependent on effluent pH.

- The combined results from all studies indicated that high pH and ammonia were the primary candidates for the cause of trout and daphnid toxicity.

- Metals were suspected as contributors to daphnid toxicity at near-neutral pH.

- Recommendations from the second treatability study were used for pH adjustment implementation at the site. Sulphuric acid, manufactured at the smelter site, is used to reduce the pH to a target of 8.8.

- The effluent had been acutely lethal to trout or *Daphnia magna* in most toxicity tests, but most recently was consistently nonlethal to both species.

- Given the amount of effort and expense involved with the TIE studies, the conclusions generated were relatively straightforward (e.g. ammonia as main cause of toxicity). However, the treatability studies provided useful information on the primary cause of effluent toxicity and also specific directions for effluent treatment and elimination of toxicity.

- Use of chemical analyses were either inadequate or poorly organized with the TIE studies.

- Logical explanations for the testing approaches used were lacking (e.g., modification to Phase I TIE, filtration prior to pH adjustment (treatability studies) , zeolite not used (TIE and treatability studies)).

3.3.4 Case Study #4 - TI/RE with Effluent from a Gold Mine

The fourth case study involves a gold mine. The effluent was transiently toxic to rainbow trout and *Daphnia magna* and the company engaged a commercial consultant/laboratory firm to investigate the cause(s) of toxicity. Following the TIE studies, the mine proceeded with investigations on effluent treatment options. Effluent treatability investigations were conducted by commercial engineering firms.

The mine discharge originates from an 800 acre tailings pond prior to treatment. The effluent is discharged periodically for 3 to 4 months periods during the summer depending on effluent chemical concentrations. All process water requirements are met by the use of recycled water

(50-70%) and a supply of freshwater from a nearby lake (30-50%). Based on the TIE and treatability studies, the mine installed a treatment plant in 1994 consisting of a reactor clarifier (including ferric/lime addition) and final pH adjustment with CO_2. In 1995 the mine re-evaluated the treatment plant and added an INCO SO_2/O_2 cyanide destruction module. Following completion of the treatment plant, the effluent remained transiently toxic to both trout and daphnids in 1996 and was consistently toxic in 1997. During the most recent discharge period, the effluent was toxic to both rainbow trout and *Daphnia magna* (~ 80% mortality was observed). The mine's environmental coordinator noted that the effluent met certificate of approval requirements and provincial chemical limits even before the installation of the treatment facility.

The environmental coordinator for the mine responded to the AETE TI/RE questionnaire, was interviewed over the telephone and provided the reports on the mine's TIE and treatability studies. After reviewing the reports, this investigation was considered a suitable candidate for a case study. The study was an appropriate selection since it was conducted fairly recently, included modified Phase I, II and III TIE studies as well as effluent treatability investigations. The study was selected after the approval of the mine's environmental coordinator, who also provided further information regarding the current status of the effluent and treatment.

Modified Phase I, II and III TIE studies and treatability investigations were initiated in 1992. Phase I, II and III TIE testing involved fathead minnows and *Daphnia magna*. The TIE studies followed the U.S. EPA protocol. Phase III TIE studies involved effluent spiking with copper and silver. Treatability investigations included bench scale evaluations to compare the available treatment methods for the effluent, water reclamation for re-use in the mill and pilot plant studies. Pilot plant studies were undertaken using information generated during the bench scale evaluations. The two most promising effluent treatment methods identified in the bench scale evaluations, lime/ferric and lime only treatments, were compared on the basis of final effluent quality. The mine reported that toxicity testing was included in bench scale evaluations and pilot plant studies.

The causes of effluent toxicity were not identified to the satisfaction of the client. In the opinion of the mine's environmental coordinator, the results of the TIE study and subsequent effluent treatability investigations were not worth the cost of the study since the substance(s) responsible for effluent toxicity were not definitively identified and effluent toxicity was not eliminated following installation of an effluent treatment plant.

3.3.4.1 Overview of the Modified TI/RE Study

The TI/RE study included education of mine personnel regarding housekeeping practices and identification of potential sources of toxicity by the mine's environmental coordinator. The consultant hired by the mine began the process with a review of the historical effluent chemical and toxicity data. Copper was identified by the consultant as the primary source of toxicity to *Daphnia magna*. Silver was also identified as a potential occasional contributor to daphnid toxicity. Copper levels also occasionally exceeded levels lethal to fish. However, data used to make these conclusions were not provided in the report and could not be evaluated or verified. The consultant noted that prior to the current study several attempts were made to collect a sample that was toxic to fathead minnows, however, all samples were nonlethal and therefore a Phase I TIE could not be conducted. From the text of the report it also appeared that selected TIE treatments with *Daphnia magna* may have been conducted and the results used to conclude that copper and silver were sources of toxicity.

The commercial laboratory/consultant indicated that at the request of the mine, a complete comprehensive report including all test results was not prepared. Instead, periodic summaries of test results were provided in letter format to the mine. With the permission of mine personnel, several telephone calls were made to the consultant requesting copies of the letter summaries, however, they were never provided. While the review may have been more complete if the summaries had been available for evaluation, the information was not considered critical to the case study since the main TIE test results were provided by the consultant.

A clear statement of the study objective was provided along with the dates of sample collection and test initiation (May - July 1992). The main objectives were as follows:

- to determine if fathead minnows and rainbow trout demonstrated similar sensitivity to the effluent thus supporting the use of fathead minnows as a surrogate species for use in TIEs,
- to provide insight into the toxicants responsible for periodic fish toxicity, and
- to address the contribution of silver and copper to *Daphnia magna* toxicity.

The consultant conducted a modified Phase I TIE study to characterize the toxicant(s). It appears that the modifications and choice of treatments were based on the consultant's review of historical chemistry and toxicity data. As indicated above, data used to make this conclusion were not provided in the report and could not be evaluated or verified. The Phase I TIE included graduated pH testing at pH 6, 7, 8 and 9; filtration at pH 10; treatment with EDTA and treatment with sodium thiosulfate. Initial untreated tests were conducted at the pH of the effluent as received (pH 8). Baseline tests were conducted so that responses observed in the effluent treatments (e.g., following the manipulations) were compared to the toxicity of an untreated sample.

The consultant referenced the U.S. EPA test protocol, but bioassay conditions were not provided in the methods section and it was initially unclear if the Environment Canada test protocols were followed, or if a modified testing approach was used. A review of raw test data (not included in the report, but provided by the consultant upon our request) revealed that each exposure concentration consisted of 10 fish (24-48 hours old) or daphnids (<24 hours old) exposed to 100 mL of either treated or untreated effluent.

Chemical analysis of the untreated sample included an ICP 29 element scan and ammonia. A summary of toxicity test results for rainbow trout, fathead minnows and *Daphnia magna* are provided in Table 3-18. Rainbow trout and fathead minnow 96 hour LC50s were comparable in the initial untreated effluent sample, therefore the consultant concluded that Phase I TIE treatments could be conducted using fathead minnows as a surrogate test species.

A review of the raw data benchsheets revealed the Phase I TIE treatments were conducted over approximately an 11 day period. Daphnid mortality was similar in the untreated initial and baseline tests, indicating storage of the effluent did not reduce daphnid toxicity. However, baseline tests conducted with fathead minnows showed a noticeable reduction in toxicity over time.

Treatment of the effluent with EDTA and filtration at pH 10 was effective in eliminating toxicity to fathead minnows and *Daphnia magna*. Treatment with sodium thiosulfate removed toxicity to fathead minnows, but had only a "slight" beneficial effect in tests with *Daphnia magna*. It was reported that daphnid mortality was reduced in samples adjusted to pH 9 compared to those adjusted to pH 6, 7 and 8. The consultants noted in the report that this was consistent with toxicity due to copper (e.g., the toxicity of copper has been shown to decrease at higher pH). Fathead minnow mortality was reduced in samples adjusted to pH 7 and 8 compared to those samples adjusted to pH 6 and 9. Increased fish mortality at lower pH was reported as consistent with copper toxicity, however, an increase in toxicity at higher pH suggested the presence of a second toxicant.

The consultant compared levels of contaminants identified in the effluent (total and dissolved measurements) with effect levels for acute toxicity (generally LC50s) as reported in the literature. Copper was the only constituent observed to exceed concentrations reported to be lethal to fathead minnows and *Daphnia magna*. Chemical analysis of the post-filtration samples revealed that total copper was reduced to a level below the lethal threshold for both species. The filtered solution was not analyzed for dissolved copper concentrations. The consultants reported that un-ionized ammonia was not expected to cause fathead minnow or trout mortality at the pH of the untreated sample (pH 8), but may contribute to toxicity if there was an increase in effluent pH or if levels increased further.

Conclusions of Phase I TI/RE Study

The consultant concluded that copper was the prime substance responsible for the toxicity to fathead minnows and *Daphnia magna*, based on the following:

- copper concentrations in untreated effluent sample were elevated above lethal threshold levels for these species,
- effluent toxicity was reduced or eliminated following treatment with EDTA, sodium thiosulfate and filtration at pH 10, and
- a reduction in the concentration of total copper below lethal threshold levels resulted in a nonlethal effluent.

The consultant also concluded that fathead minnows were an appropriate surrogate species for use in rainbow trout TIEs.

Effluent Spiking Tests

Further tests were then conducted using pH 10 filtered effluent samples spiked with copper, silver and a combination of the two metals. Even though silver was below lethal threshold levels in the TIE effluent sample, it was included in the effluent spiking tests since elevated levels had been observed in previously tested samples. Tests involving fathead minnows and daphnids were conducted under similar conditions to those used during the Phase I TIE.

Copper Spike:

Nominal and measured copper concentrations were similar. The fathead minnow and *Daphnia magna* LC50s in the spiked effluent were similar to that obtained in tests conducted on the untreated sample. The consultant reported that this data supported the conclusion that copper was responsible for the TIE sample toxicity.

Silver Spike:

Measured silver concentrations were substantially lower than the target nominal values. The consultant concluded that other effluent constituents interfered with the chemical analysis resulting in artificially low silver values. This conclusion was based on the fact that chemical analysis indicated the stock solution was accurately prepared (data was not provided in the report) and a review of documented spiking techniques indicated that the spikes were performed correctly. The consultant noted this type of interference with silver analysis had been observed in other mine effluent samples, but further details were not provided.

All LC50 values were calculated using nominal values and based on a 48 hour exposure period. The LC50 for silver based on exposure of fathead minnows to pH 10 filtered effluent was 18 μg/L, which was reported as being in agreement with literature values. The consultant reported that this data supported the conclusion that silver was not responsible for the TIE sample toxicity since effluent concentrations were below the LC50.

The nominal LC50 of silver to *Daphnia magna* in pH 10 filtered effluent was 2.1 μg/L, which was reported as being above the LC50 value in clean reconstituted water. The consultant suspected that the higher LC50 values in the gold mine effluent was a result of contaminant interactions which likely reduced silver toxicity. The consultant reported that this data supported the conclusion that silver was not responsible for the TIE sample toxicity since the concentration of silver in the effluent was below the silver LC50. However, silver concentrations measured in previously tested samples occasionally approached the LC50 for daphnids, suggesting that silver may contribute to toxicity.

Copper and Silver Spike:

Nominal and measured copper concentrations were similar, but measured silver concentrations were below target nominal values. LC50s values were in agreement with the untreated effluent sample, indicating silver did not contribute to toxicity.

Effluent Treatability Investigations

Following the Phase I TIE studies, engineering consultants hired by the mine commenced effluent treatability investigations. Bench scale testing involved investigations into potential treatment alternatives for mine tailings effluent. Treatment approaches were compared on the basis of effluent quality and preliminary estimates of capital and operating costs. Effluent quality targets were set for copper and nickel, such that the treatment method chosen would reduce these parameters below toxic thresholds for rainbow trout and *Daphnia magna*. Bench scale and pilot plant study targets for total copper, nickel and total suspended solids (TSS) were set below 0.05, 0.2 and 15 mg/L, respectively. Results from the bench scale tests revealed that chemical treatment with ferric sulphate and lime at pH 9.5, followed by clarification, was the preferred system. Treatment with lime only at pH 10.5 followed by clarification, was the second treatment choice.

Pilot plant testing was undertaken in order to; i) establish that the selected treatment method was capable of producing an effluent of the desired quality on a consistent basis, ii) provide equipment sizing information, iii) provide sufficiently large treated effluent samples for toxicity testing and sludge for chemical analysis and iv) establish optimum reagent requirements.

Evaluation of the lime-only treatment approach indicated that the process reduced soluble metals to below target levels, but was unable to reduce total nickel and TSS. Consequently, this method was not included in further testing. The lime/ferric sulphate treatment process was effective during pilot plant studies in meeting target levels for total copper, total nickel and TSS. Soluble copper and nickel levels were at or below target levels without ferric sulphate additions, indicating that total copper and nickel were associated with suspended solids in the effluent. Chemical analysis of samples submitted for toxicity testing revealed total copper levels ranged from 0.03 to 0.085 mg/L, above the target of 0.05 mg/L. Total nickel and TSS were below the target levels. The concentrations for dissolved metals were not provided. Samples of the lime/ferric sulphate treated effluent were nonlethal to trout, however, partial daphnid mortalities (20%) were observed in two samples with total copper levels of 0.06 and 0.07 mg/L.

Pilot plant studies indicated that use of an anionic polymer was necessary to achieve adequate settling rates and effluent clarity. Information on reagent addition rates, effluent feed rates, sludge generation and equipment sizing was also generated from the pilot plant studies. The final selected process was based on precipitation of copper and nickel by additions of ferric sulphate and lime at pH 9.5. Pilot plant trials indicated that the precipitate removal could be accomplished by means of a reactor-clarifier and the resulting effluent would meet the existing regulatory limits for toxicity (≤50% trout and daphnid mortality), TSS (30 mg/L), copper (0.6 mg/L) and nickel (1 mg/L).

Subsequent to the installation of the treatment facility, the mine added an INCO sulphur dioxide/air cyanide destruction module. This process is based on the oxidation of free cyanide to the cyanate ion using mixtures of sulfur dioxide and oxygen as the oxidizing agent in the presence of soluble copper. Under optimum operating conditions, total cyanide can be removed to levels as low as 0.1 mg/L and zinc, copper, iron and nickel can be reduced to less than 1 mg/L (with copper as the exception in some cases). Further details regarding the specific reactions can be found in "The draft development document for the effluent limits regulation for the metal mining sector" (Ontario Ministry of the Environment 1993).

The final selected treatment system included four technology trains:

- 800 acre tailings pond for settling and natural destruction of cyanide and metals.
- INCO cyanide destruction process.
- Lime/ferric sulphate treatment to remove metal hydroxides in a reactor clarifier (some flocculent added to assist in settling). Sludge is returned to the tailings pond.
- Carbon dioxide to adjust pH to 8.0 prior to effluent discharge.

The system currently reduces total copper concentrations to <0.1 mg/L and total nickel concentrations to <0.2 mg/L. To help further reduce ammonia loadings to the tailings pond, mine water is diverted to a separate holding pond that is recycled into process or used for firefighting and dust suppression on roads.

The total capital cost for the treatment facility was approximately $5 million dollars, with a current annual operating cost of approximately $400,000.

3.3.4.2 Comments on the TI/RE Approach

i) Approach to Phase I TIE

The consultant followed a modified Phase I approach with a focus on metals as the source of toxicity. The selected TIE treatments appeared to be based on historical toxicity data which indicated that effluent copper levels occasionally exceed lethal thresholds.

As indicated earlier, the consultants did not provide all of the letter summaries and thus it was not possible to determine if the selection of treatments was logical. As noted in case study #1, it is possible to overlook classes of compounds that contribute to toxicity if Phase I is modified. This particular concern was noted by the mine's environmental coordinator who felt that the TIE study lacked focus and did not include an investigation into toxicity due to organic complexes. However in the sample collected for TIE testing, complete removal of toxicity with EDTA and filtration at pH 10 is indicative of toxicity due to the presence of metals. Furthermore, the effluent spiking experiments supported the hypothesis that copper was the main substance contributing to toxicity. It is important to note that this conclusion was only applicable to the sample currently under investigation. Repeat testing, including toxicity and chemical analysis, is generally recommended to confirm the sources of toxicity.

Additional treatment of the effluent involving the use of cation exchange resins during the TIE study would have provided supporting evidence that metals were the main source of toxicity. The advantage of exchange resins is that the toxicant can be removed from solution and then recovered from the resin. If toxicity is reduced following treatment with cation exchange, the toxicants can be recovered from the resin and re-tested for toxicity. The toxicity results and chemical analysis of the recovery solution, untreated and treated effluent are compared to determine the cause of effluent toxicity.

It was reported by the consultant that daphnid toxicity was reduced in effluent samples adjusted to pH 9 compared to samples adjusted to pH 6, 7 and 8. However, a review of the raw data revealed that adjustment to pH 9 only slightly delayed mortality and was considered in this evaluation to have no meaningful effect on toxicity. After 4.5 hours of exposure, complete daphnid mortality was observed at pH 6, 7 and 8, whereas 60% mortality was observed at pH 9 and the remaining organisms were reported as immobile. At 23 hours, complete mortality was observed in all exposure solutions. A similar response was reported for fathead minnows. The consultant reported that fathead minnow toxicity was greater at pH 6 and 9 compared to pH 7 and 8. In this case, fathead minnow mortality was substantially delayed at pH 7 and 8. After 20 hours of exposure, 100% mortality was observed at pH 9, 60% mortality at pH 6 (remaining organisms were reported as stressed) and 0% mortality at pH 7 and 8 (organisms were reported as stressed). At 23 hours, complete mortality was observed in all exposure solutions. The main point of this example was to emphasize that a complete report, including all raw data, can be useful for mine personnel to evaluate treatment results.

The consultant provided a thorough summary of both total and dissolved metal concentrations in the initial untreated effluent sample. However, TIE investigations continued with the same effluent sample for approximately 11 days and supporting chemical analysis of the stored effluent was not provided. Information on changes in effluent chemistry were particularly important given the narrow focus of the TIE study and since results from the untreated baseline tests with fathead minnows indicated that toxicity was reduced overtime.

ii) Effluent Spiking Experiments

The spiked effluent tests supported the conclusion that copper was responsible for the TIE sample toxicity. However, tests involving silver were difficult to interpret and of limited value since measured and nominal values were not in agreement. The consultants hypothesized that silver was less toxic in the mine effluent than in clean reconstituted water because of contaminant interactions in the whole effluent. However, the effluent composition and contaminant interaction apparently had no effect on fathead minnow LC50s since there was agreement with literature values. In fact, LC50s for silver in the mine effluent were not determined. The consultant did note that further investigations would be required to determine the specific role of silver in mine effluent.

iii) Use of Fathead Minnows

Based on a comparison of 96 hour LC50s for trout and fathead minnows in untreated effluent, the consultant concluded that fathead minnows could be used as surrogate TIE test species in place of rainbow trout. However, all subsequent tests were based on 48 hour exposures, even though trout and fathead minnow results were not comparable at 48 hours (Table 3-18). No explanation was provided as to why 48 hour LC50s were used over 96 hour LC50s. Furthermore, the decision to proceed with fathead minnows appeared to be based on a single toxic effluent sample. At a minimum, testing must be conducted prior to TIE test initiation to determine that the species of interest and the surrogate species respond in a similar manner to the untreated effluent under a variety of conditions. As in case study #1, the rainbow trout was the regulatory species of concern, yet fathead minnows were used in this TIE study. The use of surrogate test species is often necessary for those TIE treatments which are limited by the ability to treat only small effluent volumes (e.g., treatment with C18). The use of rainbow trout generally necessitates greater effort and expense since trout require larger test volumes. However, based on the consultant's effort to streamline the TIE, the limited number of treatments could have been easily and economically conducted without the use of a surrogate species. This would have been of more value to the client in the Canadian context.

iv) Lack of statistics

The consultant's report did not include a description of how the LC50s were calculated, nor is there any mention of confidence limits for these parameters. It would have also been preferable if the data had been compared statistically to allow a more rigorous definition of "slight" reductions in toxicity. For example, treatment with EDTA was reported to have reduced toxicity, when in fact a review of raw data (not provided to the mine client) clearly indicated that selected EDTA concentrations completely eliminated toxicity. Similarly, sodium thiosulfate was reported to have "slightly" reduced daphnid toxicity, yet the raw data indicated that the addition of sodium thiosulfate significantly reduced effluent toxicity. LC50s and confidence limits (for selected samples) were calculated during the review of this case study using raw data provided by the consultant. The untreated effluent LC50 (95% confidence limits) was 5% (3.8-6.6) and thiosulfate addition increased the LC50 (95% confidence limits) to 29% (16.7 - 44.7).

v) Effluent treatability studies

Toxicity testing with rainbow trout and *Daphnia magna* was included in all bench scale and pilot plant investigations to ensure that the selected treatment would successfully eliminate acute lethality. The engineering firm conducting the pilot plant studies indicated that the chemical targets were such that the treatment method chosen would reduce copper, nickel and TSS to below toxic thresholds for rainbow trout and *Daphnia magna*. However, the target values were based on total metal concentrations with apparently little emphasis on dissolved concentrations which are the most relevant in terms of toxicity. In fact, the copper target selected by the engineering firm (0.05 mg/L) exceeded the LC50 for *Daphnia magna* (0.044 mg/L) as reported in the TIE study. Daphnid toxicity may be observed at the target level if a large percentage of the total copper is actually present in the dissolved form. For example, partial daphnid mortalities (20%) were observed in two samples with total copper levels of 0.06 and 0.07 mg/L, but not in samples containing 0.085 mg/L total copper. The concentration of dissolved copper was not provided for these tests. However, chemical data generated during the pilot plant study indicated that the dissolved copper in the treated effluent ranged from 0.01 to 0.05 mg/L (note that dissolved measurements were only included for a limited number of samples). It should be noted that the pH of the treated effluent can also affect the solubility (and therefore the bioavailability) of metals.

It appears that the copper target may have been selected from the chemical analysis of the filtered effluent at pH 10 which reduced total copper concentrations to 0.04 mg/L and completely eliminated daphnid toxicity. However, the filtered sample was only analyzed for total copper and the dissolved copper concentration was unknown. It could not be assumed that the copper concentration measured in the Phase I pH 10 filtered effluent represented the dissolved concentration. A 1.0 μm filter is used in the Phase I filtration procedure, rather than the "standard" 0.45 μm filter used to distinguish between dissolved and particulate substances. Based on the above information, the partial daphnid mortalities observed during the pilot plant studies and after full treatment plant implementation may have been explained by the concentration of dissolved copper.

A complete review of the current effluent toxicity and chemistry data would be required to determine why the treatment plant was not effective at eliminating toxicity. Effluent collected during the pilot plant trials indicated all treated samples were nonlethal to trout, however, partial daphnid mortalities (20%) were observed in those samples that exceeded the total copper target levels.

3.3.4.3 Overall Summary of Case Study

- The client, a gold mine, did not feel the results from the TI/RE studies were worth the cost since the effluent remained acutely lethal even after a full effluent treatment facility had been installed.
- The general characteristics of the suspected toxicant(s) were identified (e.g., metals - most likely copper), but were not confirmed using the TIE process.
- Treatability investigations included bench scale evaluations, water reclamation and pilot plant studies. A full effluent treatment plant was installed based on the results of the treatability studies. However, the target level for copper used during these studies was at the *Daphnia magna* LC50.
- Effluent tested during the pilot plant trials indicated all treated samples were nonlethal to trout. However, partial daphnid mortalities (20%) were observed in those samples that exceeded the total copper target levels.
- During the most recent discharge period, the effluent was toxic to both rainbow trout and *Daphnia magna* (~ 80% mortality was observed). Ammonia, produced during the destruction of cyanide, is the suspected cause of trout mortality. Metals may be the cause of daphnid toxicity.

Table 3-18. Responses of rainbow trout, fathead minnows and *Daphnia magna* during modified Phase I TIE involving effluent from a gold mine

Treatment	Rainbow trout	Fathead minnow	***Daphnia magna***
initial tests (pH 8) - 3 day old effluent	LC50 (48 hr) = 23% LC50 (96hr) = 21%	LC50 (48 hr) = 57% LC50 (96hr) = 26%	LC50 (48 hr) = 5% LC50 (96hr) = 4%
Baseline test - 11 day old effluent (48 hour LC50)	-[a]	LC50 = 100%	LC50 = 5%
Graduated pH (pH 6)	-	no effect	no effect
Graduated pH (pH 7)	-	delayed mortality	no effect
Graduated pH (pH 8)	-	delayed mortality	no effect
Graduated pH (pH 9)	-	no effect	no effect
Filtration at pH 10	-	nonlethal	nonlethal
Sodium thiosulfate	-	nonlethal	LC50 = 29.3 (reported by consultant as a "slight" reduction in toxicity)
EDTA	-	nonlethal (reported by consultant as reduced toxicity)	nonlethal
Chemical analyses	yes	yes	yes

[a] not tested

3.3.5 Case Study #5 - TIE With Effluent From a Cobalt/Nickel and Precious Metals Refinery

The fifth case study involved a cobalt/nickel and precious metals refinery. The effluent was transiently toxic to rainbow trout and *Daphnia magna* and the company engaged two commercial consultant/laboratory firms to investigate the cause(s) of toxicity over a period of 3 years. Several Phase I TIE studies were completed and Phase II and III studies are being evaluated.

Two main processes occur at the refinery operation: cobalt/nickel refining and precious metal refining. The effluent treatment plant (ETP) treats all of the wastewater streams from the metal refining/processing operations and their associated support services, surface run-off from the 360 acre site and water from groundwater recovery projects. The ETP also receives run-off from two local subdivisions and wetland drainage. Prior to entering the ETP, the wastewater is automatically adjusted to pH 7.5 to 8 using sulphuric acid to control influent pH. A coagulant is added to the raw water as it is pumped to one of two 70-foot diameter reactor clarifiers. The raw water is then automatically adjusted with slaked lime ($Ca(OH)_2$) slurry to a pH of approximately 10.6 to precipitate the metals. A polymer is added to flocculate and settle the solids. The clarified water overflows to a launder and is automatically adjusted with carbon dioxide to achieve a pH not greater than 9.5 at the regulated control point. The treated effluent, with an average flow of less than 20,000 cubic meters per day, discharges into the polishing pond before flowing past the regulated control point and entering the receiving water.

The environmental coordinator responded to the AETE TI/RE questionnaire, was interviewed over the telephone and provided the reports on the mine's TIE studies. After reviewing the reports, this investigation was considered a suitable candidate for a case study. The study was an appropriate selection since it was conducted fairly recently and included Phase I and II TIE studies. The study was selected after the approval of the environmental coordinator, who also provided further information regarding the current status of the effluent and treatment.

Two different consultant/commercial laboratory firms were contracted to investigate the cause of effluent toxicity. The studies were conducted between 1996 and 1998. Testing involved fathead minnows, rainbow trout and *Daphnia magna*. Several causes of toxicity were suspected, but not identified using the traditional Phase I TIE treatments.

In the opinion of the environmental coordinator, all investigations were useful in the characterization of the suspected toxicant(s). However, it was noted that the "standard" approach to toxicant identification was not effective. The development of novel methodologies and techniques will be required for future investigations.

3.3.5.1 Overview of the 1996/97 TI/RE Studies

A clear statement of the study objectives was provided along with the dates of sample collection and test initiation (March - June 1996). The main objectives were as follows:

- thoroughly characterize effluent toxicity and chemistry,
- relate effluent toxicity to specific effluent constituents,
- associate variability in effluent toxicity and chemistry with cycles in plant processes, and
- undertake Phase I TIEs on at least two toxic samples to ascertain the general characteristics of the effluent toxicant(s).

The consultant began the TI/RE process with a review of the historical chemical data and effluent toxicity data. Chemical analysis (unfiltered samples) of the raw effluent samples included cations (ICP 29 element scan), anions and ammonia. It was noted that none of the individual measured chemical parameters exceeded published LC50 values for rainbow trout, *Daphnia magna* or fathead minnows. The only possible source of toxicity suggested during the review was the high concentration of total dissolved solids (TDS). Initial testing involved daily collection and testing of samples over a two week period during March 1996. The tests involved exposing rainbow trout and *Daphnia magna* to the full strength (100%) effluent. The results indicated that the samples were least toxic at the beginning of the week because of the weekend shut-down and the 1-2 day lag period. The samples were also observed to be more frequently toxic to *Daphnia magna* than rainbow trout.

1996 Phase I TIE Studies

Several Phase I TIE studies were conducted on samples collected in 1996 using rainbow trout, *Daphnia magna* and fathead minnows. It should be noted that fathead minnows were used as a surrogate test species even though in most cases the effluent was not toxic to rainbow trout. The consultant referenced the U.S. EPA guidance document, but bioassay conditions (e.g., exposure volumes, number of test replicates etc.) were not provided in the any of the reports. In addition to the "standard" U.S. EPA treatments, zeolite was included in the Phase I TIE. Raw data was not provided in the reports for any of the Phase I treatments. Rather, results from each treatment were presented as having "no effect", "reduced" or "removed" toxicity.

The first sample submitted for a Phase I TIE manipulation (March 10, 1996) resulted in complete *Daphnia magna* mortality, but was nonlethal to rainbow trout and fathead minnows. Zeolite was the only treatment that removed toxicity. Chemical analysis of the post-zeolite treated effluent was not conducted. The consultant noted that (total) copper was present in the untreated effluent at concentrations slightly exceeding the reported LC50 for *Daphnia magna* (0.04 mg/L). The total ammonia concentration (<0.05 mg/L) was reported as well below the level that would cause daphnid toxicity.

Phase I testing was conducted on a second sample (March 17th) which resulted in complete mortality of *Daphnia magna* and rainbow trout. Although the sample was nonlethal to fathead minnows, testing proceeded using daphnids and minnows since the fish were completely immobilized in the full strength effluent at test completion. The daphnid TIE results were similar to the March 10th sample where toxicity was removed by treatment with zeolite. Toxicity to fathead minnows was also removed by aeration at pH 3 and treatment with zeolite. A slight reduction in toxicity was observed following filtration at pH 3, treatment with EDTA and sodium thiosulfate. The consultants noted that the removal of toxicity by zeolite suggested that ammonia or metals were responsible for the observed mortality. However, as noted during the data review, ammonia was not a likely cause of effluent toxicity based on the low measured concentrations (<0.05 mg/L). The slight removal of toxicity by treatment with EDTA and sodium thiosulfate also suggested toxicity due to metals, but the lack of complete removal suggested the presence of a secondary toxicant. Removal of toxicity by aerating the effluent at pH 3 was suggested to be indicative of an acid-labile or acid-volatile toxicant. Samples of the post-zeolite or pH 3 aerated effluent were not submitted for chemical analysis.

The consultant noted that TDS was elevated in the samples tested (TDS values were not provided in the report) and used a computer model to predict whether the dissolved solids concentrations were sufficient to account for the observed mortalities. The model predicted that the TDS concentration was sufficiently high to cause daphnid mortalities. The model was referenced in the report (Mount and Gulley, 1992), but the raw data from the model was not provided in the report and therefore the methods used or the conclusions drawn could not be evaluated.

Conclusions of 1996 Phase I TI/RE Studies

The specific identity of the toxicant remained unknown, however, several key pieces of information were generated during the Phase I TIE testing:

- toxicity was cyclic and appeared to be correlated with on-site processes.
- the effluent was typically more toxic to *Daphnia magna* than to rainbow trout.
- the primary toxicant was not volatile, filterable, affected by radical pH adjustment alone, a non polar organic, an oxidant nor a cationic metal; the secondary toxicant may have been a metal(s).
- zeolite removed toxicity to *Daphnia magna*; zeolite and aeration at pH 3 removed toxicity to fathead minnows.
- TDS may have been the cause of toxicity to *Daphnia magna*.

Based on these results, the consultant suggested that testing continue until a sample lethal to trout was obtained for Phase I TIE testing. However, the samples remained nonlethal to trout and the program was temporarily halted. Testing continued in July 1996 immediately after temporary plant shut-down. Modelling of the data conducted by the consultants (data not provided in the report) confirmed that *Daphnia magna* toxicity was caused by high TDS. The dissolved solids of interest were sodium, potassium, calcium, magnesium, chloride, sulphate and bicarbonate. The concentrations of these ions increased as production increased. The concentration of copper also increased to levels expected to be toxic to *Daphnia magna* (0.04 mg/L) yet many of these samples were nonlethal. It must be noted that only the total metal concentration was analyzed and the dissolved copper concentration was unknown.

1997 Phase I TI/RE Studies

Additional testing was undertaken in 1997 to further characterize and identify the substances responsible for toxicity. Testing included analysis of process streams, simulated effluent assessments and additional Phase I TIEs

The main process effluents (a precious metals refinery and cobalt refinery) and other contributors to the final effluent (wetlands discharge) were sampled and tested for toxicity. The cobalt stream sample exhibited the greatest toxicity to daphnids (LC50=9%) and trout (LC50=18%). The precious metals refinery sample was also highly toxic to daphnids (LC50=14%), but was nonlethal to trout. The wetlands sample was nonlethal to both species. It was concluded that the cobalt refinery represented the largest potential source of trout toxicity, while the cobalt and precious metals refinery both contributed to daphnid toxicity. Based on the process stream test results, a Phase I TIE was conducted on the cobalt process stream using *Daphnia magna* and fathead minnows. Toxicity was not reduced by any treatment (including zeolite). It was concluded that toxicity was a result of elevated TDS or that the elevated TDS levels were masking the effects of an unidentified "other" toxicant. Additional treatments that had been previously shown to reduce daphnid and fathead minnow toxicity (zeolite, aeration at pH 3) were conducted on the cobalt effluent using rainbow trout. Both treatments were ineffective at reducing toxicity and it was suggested that the trout mortality was also related to TDS.

The consultant attempted to simulate the TDS content of toxic effluent samples by adding appropriate amounts of selected dissolved solids (e.g., sodium, potassium, calcium, magnesium, chloride, sulphate and bicarbonate) to de-ionized water. All toxicity was accounted for in the simulated effluent tests and it was therefore concluded that high TDS was the main cause of daphnid mortality. Details regarding the specific experiments were not provided and therefore could not be evaluated during this review.

A Phase I TIE was initiated on a final effluent sample collected on October 20, 1997. The sample was acutely lethal to fathead minnows, rainbow trout and *Daphnia magna*. All treatments were conducted using rainbow trout and fathead minnows. Zeolite was the only treatment to remove toxicity to both species. Activated carbon eliminated effluent toxicity to rainbow trout, but only partially reduced fathead minnow mortality. Chemical analysis indicated that TOC and DOC concentrations decreased after treatment with carbon and zeolite. It was suggested that the decrease in TOC/DOC indicated that the toxicant was an organic or organometallic compound.

Additional tests were undertaken on the October 20th sample to clarify the effect of zeolite on the removal of toxicity. The tests included an unsuccessful attempt to extract the toxicant from the zeolite column using NaOH and $CaSO_4$. Chemical analysis of the post-zeolite treated effluent was conducted during these investigations. However, at this point it was decided that the study approach would be re-evaluated and the results of the post treated zeolite sample would be interpreted at a later date.

Conclusions of 1997 Phase I TI/RE Studies

- fathead minnows were more sensitive to effluent dissolved solids than rainbow trout and therefore should not be used as a surrogate test species.
- adjustment of samples to pH 3 resulted in sufficient additional dissolved solids to increase the toxicity of the effluent when total dissolved solids were already elevated, potentially making this treatment ineffective.
- computerized modelling confirmed that TDS was the main cause of *Daphnia magna* and fathead minnow toxicity.
- an organic or organometallic compound was the suspected cause of trout toxicity.
- any further testing would require the use of novel separation techniques.

3.3.5.2 Comments on the 1996/97 TI/RE Approach

The initial stages of the TI/RE appeared to have been conducted in a logical manner. For example, the consultant reviewed the available toxicity data to determine if there were any obvious causes of effluent toxicity. The review was beneficial since it immediately eliminated ammonia as a potential cause of toxicity. The consultant provided a thorough assessment of the process streams that comprise the final effluent discharge. Effluent treatment chemicals were also reviewed as a potential source of toxicity.

i) Approach to Phase I TIE

The consultant followed the standard Phase I TIE approach, incorporating the use of zeolite at the first stage of testing. The consultant attempted novel extraction techniques with NaOH and $CaOH_4$ to recover the substance responsible for toxicity from the zeolite column. It is important to note that cation exchange selectivities in zeolites do not follow typical rules and patterns exhibited by traditional ion exchangers. Zeolites are crystalline aluminosilicates and will not only act as ion exchange columns, but also exhibit high selectivity for those ions which will easily enter the zeolite pores (Sherman, 1978). These differences in cation exchange behaviour explain why the attempts to recover the suspected toxicant(s) using sodium hydroxide and calcium sulphate were not successful. Novel columns techniques were also used, however, the specific columns and methods used were never identified. It was reported that none of the columns were effective at reducing toxicity and that the results would be provided in a later report.

A complete comprehensive report was not prepared for the client. Instead, periodic summaries of test results were provided in letter format. Consequently, it was not possible to fully evaluate the test results during this review since the raw data were not provided for any of the Phase I tests. Furthermore, test conditions (e.g., exposure volumes, number of organisms per replicate) were not described in any detail in the reports. The use of a computer model to predict if the concentration of dissolved solids could account for the observed daphnid toxicity appeared to be a useful approach and involved the use of newly developed techniques. However, the specific results were not provided in the report and could not be evaluated during this review.

Zeolite was the only treatment which effectively eliminated toxicity. Although this information was available early in the Phase I studies, chemical analysis of the post zeolite treated effluent was not conducted until 1.5 years after the investigation had been initiated. Samples of effluent should have been analysed before and after treatment with zeolite, particularly since none of the traditional Phase I treatments were as effective at reducing effluent toxicity. Identification of the specific causes of effluent toxicity during the 1996/97 studies were also confounded by the fact that all chemical analyses were based on total rather than dissolved chemical parameters. While particulate bound chemicals can be a source of toxicity (e.g., through ingestion), dissolved (water soluble/hydrophilic) chemicals are more readily available to the organisms of interest and are consequently the most relevant in terms of toxicity.

ii) Use of fathead minnows

Fathead minnows were used in place of the key species of interest (rainbow trout) even though effluent samples were only occasionally lethal to trout and yet consistently toxic to fathead minnows. This resulted in the generation of data that was applicable to fathead minnows, but could not be used to explain trout mortality. In fact, the consultant noted in the final letter summary to the mine that fathead minnows were not an adequate surrogate for trout. However, this should have been obvious from the very first set of tests where the effluent was toxic to fathead minnows, but was nonlethal to trout. The difference in response suggested that the organisms were responding to different toxicants or differed in their sensitivity to the same toxicant. Regardless of which explanation was correct, the surrogate and regulatory species did not respond similarly to the untreated effluent and therefore no justification existed for the use of fathead minnows.

iii) Lack of statistics

Statistical analysis of the data was non existent. It is unknown why the chemistry or toxicity data were not analyzed statistically given the large amount of data generated during the two year study. As mentioned in several of the other case studies, the lack of statistical comparisons may not be critical in most TIE work, where gross changes in toxicity are the only consideration. However, this study included the testing of numerous samples collected over a two year period, and all samples were tested for toxicity and analysed for a battery of chemical parameters.

In addition, the report mentions that toxicity was "reduced" after certain treatments. It would be preferable if the actual data had been provided and (if possible) compared statistically to account for the variation in effluent and sample toxicity over time and to allow a more rigorous definition of "reduced" toxicity. At a minimum, the percent mortality or LC50 values should have been presented in the data reports to allow the client to fairly evaluate the effect of any given treatment.

3.3.5.3 Overview of the 1998 TI/RE Studies

The consultant began with a review of the historical chemical and toxicity data. The refinery had experienced sporadic failures of laboratory effluent toxicity tests for several years and the historical data indicated that, in recent years, the effluent has been more frequently lethal to *Daphnia magna* than to rainbow trout. The test data also indicated that effluent quality was variable and that mortality often coincided with high TDS. The measurement of conductivity (which is reflective of TDS) had generally been greater than 7,000 µS/cm for samples lethal to *Daphnia magna* and greater than 12,000 µS/cm for samples lethal to rainbow trout. This information was used to predict effluent toxicity and reduce the cost of needless sampling.

Previous testing conducted in 1996 and 1997 suggested that the suspected toxicant(s) were atypical for an effluent associated with mining in Canada. It was suspected that elevated total dissolved solids (TDS) may have been the cause of toxicity to daphnids. However, previous testing failed to identify which ions best explained toxicity.

A review of the 1996/97 data was also conducted by a researcher at a local university. It was suggested that trout and daphnid mortalities resulted from large imbalances between sodium (Na^+) and potassium (K^+) or between sodium and calcium (Ca^{2+}). Organisms exposed to sodium enriched effluent may have been unable to maintain the appropriate cellular ratios of Na^+ to K^+ or Ca^{2+}. Although the hypothesis was not confirmed, it was felt that there was sufficient data to indicate that severe organism stress would be expected when the effluent Na/K and Na/Ca ratio was very large. Mortality in untreated effluent samples appeared to occur at Na/(Ca+K) ratios of 80:1, while the ratio decreased to approximately 30:1 in nonlethal zeolite treated samples.

The 1998 TIE study was undertaken for several reasons: i) the effluent remained frequently acutely lethal to *Daphnia magna* and occasionally lethal to rainbow trout and ii) the specific cause of effluent toxicity had not been identified in the 1996/97 studies. A clear statement of the study objective was provided along with the dates of sample collection and test initiation (March - May 1998). The main objectives were as follows:

- conduct a Phase I TIE using rainbow trout and *Daphnia magna,*
- assess the reasons for the reduction/elimination of effluent toxicity following effluent manipulation using zeolite, and
- assess the effects of changing the ratio of Na/(Ca+K) on effluent toxicity.

Samples were collected over a three month period beginning in March 1998. Each set of samples was subjected to a battery of physical and chemical treatments in an attempt to identify the substances responsible for toxicity. Specific testing included Phase I TIEs, treatment with zeolite resin and ion balance experiments (e.g., additions of KCl, $CaCl_2$ or NaCl). Chemical analysis for metals, anions, TDS, alkalinity (total and dissolved forms) was conducted on all untreated effluent samples and on any treated sample observed to reduce or eliminate toxicity. Selected treatments that did not eliminate toxicity were also submitted for analysis. All raw data for the untreated and treated effluent samples were provided with the report. Details regarding the bioassay test conditions, treatment methodology and testing approach were also provided.

Phase I TIEs

i) Daphnia magna Testing

The first untreated effluent (initial test) was acutely lethal to *Daphnia magna*. The untreated baseline tests produced results similar to those of the initial untreated tests (100 % mortality). Initial tests were also conducted using rainbow trout, however, the untreated effluent was not acutely lethal (0% trout mortality). It was noted in the report that *Daphnia magna* behaviour was variable during the 48 hour exposure to the untreated effluent. For example, at different times during the test the daphnids would appear immobilized at the bottom of the test container and then later resume moving throughout the effluent only to be noted as immobile in the next observation. The TIE results confirmed that toxicity was reduced following treatment with zeolite. The consultant noted that ammonia was not the likely cause of toxicity based on the low effluent concentration (<0.05 mg/L).

The consultant compared the concentrations of selected (dissolved) effluent constituents in the untreated effluent to toxicity data reported in the literature for *Daphnia magna* to assess if any of the measured parameters could account for all or part of the observed toxicity. The comparisons indicated that sodium was at a concentration high enough to account for approximately 50% of the mortality (sodium LC50 = 2340 mg/L). Similarly, dissolved copper concentrations approached the LC50 for *Daphnia magna* (copper LC50 = 0.04 mg/L). Other parameters were below concentrations reported to cause toxicity.

The consultant noted that changes in several chemical parameters were observed when the untreated effluent was compared to the zeolite and carbon treated samples. Carbonate, bicarbonate, cobalt, copper and nickel concentrations decreased after treatment with zeolite. A slight decrease in sodium concentration was also observed. Calcium, potassium, magnesium and strontium concentrations increased following zeolite treatment. In comparison, treatment with activated carbon resulted in a decrease in effluent concentrations of cobalt, copper, nickel and boron and an increase in the concentration of carbonate, bicarbonate and strontium. Compared to the untreated effluent samples, the concentrations of chloride and sulphate were relatively unchanged following treatment with both carbon and zeolite. TDS remained relatively unchanged following treatment with carbon and zeolite. A summary of the changes in chemical concentrations are provided in Table 3-19.

The observed increase in calcium, potassium and magnesium concentrations following treatment with zeolite agreed with previous testing conducted in 1997. In comparison, calcium, potassium and magnesium were unchanged after treatment with activated carbon. Concentrations of sodium were reduced to slightly below the LC50 to *Daphnia magna* in the zeolite treated effluent samples, but were unchanged in the carbon treated sample. Treatment with activated carbon was more effective than zeolite at reducing trace metal (e.g., cobalt, copper, nickel) concentrations. TOC (Total Organic Carbon) and DOC (Dissolved Organic Carbon) concentrations also decreased after treatment with carbon, but not with zeolite. Yet, activated carbon had little effect on *Daphnia magna* survival, while treatment with zeolite resulted in a decrease in mortality.

ii) Rainbow trout Testing

The second untreated effluent sample was acutely lethal to rainbow trout. LT50s (e.g., time when 50% of the trout were dead) were provided for all trout tests. The LT50 for the untreated initial test was 18 hours and complete (100%) mortality was observed in 43 hours. The untreated baseline tests produced similar results. It should be noted that toxicity of the effluent to *Daphnia magna* was not evaluated on this sample.

The TIE results indicated that toxicity was eliminated following treatment with zeolite. The consultant again noted that ammonia was not the likely cause of toxicity based on the low effluent concentration (<0.05 mg/L). Toxicity was also eliminated following treatment with carbon, suggesting that the toxicant may be a metal or an organic substance. A slight reduction in toxicity was observed at pH 11 in the filtration test using a 1.0 µm filter, suggesting that the toxicant(s) may be a metal. However, the general ineffectiveness of EDTA and sodium thiosulfate, suggested that the toxicant(s) was not a metal or an oxidative substance (e.g., chlorine, bromine).

The consultant compared the concentration of selected (dissolved) effluent constituents in the untreated effluent to toxicity data reported in the literature for rainbow trout to assess if any of the measured parameters could account for all or part of the observed toxicity. The comparisons indicated that the measured copper concentration was above the LC50 range reported for rainbow trout at a similar hardness (copper LC50 = 0.02 - 0.05 mg/L at hardness <100 mg/L). Other parameters (for which toxicity data was available) were below concentrations reported to cause toxicity.

It was noted that changes in several chemical parameters were observed when the untreated effluent was compared to the zeolite and carbon treated samples. Carbonate, bicarbonate, cobalt, copper, nickel and boron concentrations decreased after treatment with zeolite. Calcium, potassium, magnesium and strontium concentrations increased after treatment. A slight decrease in sodium and boron concentrations was also observed. Effluent concentrations of TDS, chloride and sulphate were relatively unchanged. A summary of the changes in chemical concentrations is provided in Table 3-20.

In comparison, treatment with activated carbon resulted in a decrease in effluent concentrations of cobalt, copper, boron and carbonate. Effluent concentrations of TDS, chloride, sulphate, calcium, potassium, magnesium and bicarbonate were relatively unchanged following carbon treatment. It was noted that the observed increase in calcium, potassium and magnesium concentrations following treatment with zeolite agreed with the results obtained during testing with *Daphnia magna*. Calcium, potassium and magnesium were unchanged after treatment with activated carbon. Similarly, treatment with activated carbon was more effective than zeolite at reducing trace metal (e.g., cobalt, copper, nickel) concentrations. TOC (Total Organic Carbon) and DOC (Dissolved Organic Carbon) concentrations also decreased after treatment with carbon, but not with zeolite. Treatment with carbon and zeolite both effectively eliminated toxicity and reduced the measured copper concentrations to less than the Method Detection Limit (MDL) (0.005 mg/L) in the case of carbon and to 0.057 mg/L in the case of zeolite.

Zeolite Treatments

Daily effluent sampling was conducted for a 7 day period. All samples were treated in duplicate with zeolite and tested using both rainbow trout and *Daphnia magna.* All untreated samples (initial tests) were acutely lethal to *Daphnia magna* (80-100% mortality). Only two of the seven samples resulted in trout mortality (10% and 50% mortality, respectively). Treatment with zeolite eliminated toxicity to both species.

The concentrations of selected (dissolved) effluent constituents in the untreated effluent were compared to toxicity data reported in the literature for both rainbow trout and *Daphnia magna.* The comparisons indicated that the measured copper concentrations were within the LC50 range reported for *Daphnia magna* for all samples (copper LC50 = 0.04 mg/L). In all but one sample, the concentration of sodium was also within the LC50 range reported for *Daphnia magna* (sodium LC50 = 2340 mg/L). A decrease in other effluent parameters was also observed in this sample (e.g., alkalinity, TDS, sulphate, calcium, potassium, magnesium and boron). The decrease in the concentration of these parameters coincided with a slight decrease in daphnid mortality; 80% mortality in untreated sample compared to 100% mortality in all other untreated samples.

The comparisons indicated that the dissolved copper concentrations for all samples tested during the continuous zeolite sampling (0.041 - 0.056 mg/L) were within the LC50 range reported for rainbow trout (copper LC50 = 0.02 - 0.09 mg/L). However, the only sample to cause significant mortality (50%) occurred at a copper concentration of 0.042 mg/L, while other samples with higher copper concentrations were nonlethal. However, the consultant noted that alkalinity (carbonate and bicarbonate) was highest in the untreated sample that resulted in 50% trout mortality. The pH of this sample (9.4) was also higher compared to any other received for testing. Although there did not appear to be any direct relationship between copper concentrations and toxicity, there may have been other modifying factors present (e.g., carbonates, pH) which may have caused the copper to impart some toxic effect. Note that the carbonate concentration decreased following treatment with zeolite. All other parameters were below the available LC50 values for rainbow trout.

As observed during the rainbow trout and *Daphnia magna* Phase I TIEs, changes in several chemical parameters were observed when the untreated effluent samples were compared to the zeolite treated samples. Carbonate, bicarbonate, cobalt, copper and nickel concentrations decreased after treatment, while calcium, potassium, magnesium and strontium concentrations increased. Effluent concentrations of chloride, sulphate and boron were relatively unchanged and sodium appeared to decrease only slightly after treatment.

Ion Balance Experiments

These tests involved obtaining toxic samples of effluent and adding varying amounts of calcium (as calcium chloride) and potassium (as potassium chloride). These chemical additions were expected to reduce the Na/(Ca+K) ratio resulting in a less toxic effluent (e.g., sodium is kept constant, while calcium and potassium increase). These experiments were run concurrently with both the *Daphnia magna* and rainbow trout Phase I TIEs.

i) *Tests with Daphnia magna*

The highest addition of calcium chloride and potassium chloride at a Na/(Ca+K) ratio of 15:1 resulted in a reduction in daphnid mortality. The Na/(Ca+K) ratios of 40:1 and 88:1 had little effect on overall survival (Table 3-21). However, observations made by the consultant during the bioassays indicated that the daphnids in the "medium" Na/(Ca+K) ratio of 40:1 appeared to remain mobile longer (~ 22 hours) compared to those in the untreated sample or in the "high" 88:1 ratio (~ 2 hours). While the Na/(Ca+K) ratio of 15:1 reduced daphnid mortality, the surviving organisms were reported as small in size and pale in colour at test completion.

A decrease in the concentration of dissolved metals (e.g., copper, cobalt) was noted as the Na/(Ca+K) ratio decreased (more calcium and potassium, less dissolved metals). For example, the concentration of dissolved copper was reduced to slightly below the LC50 for daphnids in the "low" Na/(Ca+K) ratio, but not in the "medium" or "high" ratios. The consultant noted that the chemical results were based on samples collected at the start of testing. At test completion, a small amount of precipitate was observed at the bottom of the test containers in all solutions to which calcium and potassium was added. The calcium and potassium solutions appeared to be completely dissolved at the start of testing and presence of precipitates was not noted until test completion. The consultant suggested that it was reasonable to assume that the concentration of dissolved metals would have been further reduced over the course of testing as the metals precipitated.

ii) Tests with Rainbow trout

Calcium and potassium chloride additions resulted in a reduction in rainbow trout mortality (Table 3-22). The "low" Na/(Ca+K) ratio of 15:1 resulted in slight (10%), but not significant, trout mortality. The "medium" Na/(Ca+K) ratio of 60:1 was nonlethal to trout. The "high" Na/(Ca+K) ratio (smallest amount of calcium and potassium added to the effluent) resulted in 30% trout mortality.

As noted during the *Daphnia magna* tests, a decrease in the concentration of dissolved metals (e.g., copper, cobalt, nickel) was noted as the Na/(Ca+K) ratio decreased (e.g., more calcium and potassium, less dissolved metals). The concentration of dissolved copper was reduced to below the LC50 for trout in the "low" Na/(Ca+K) ratio, but not in the "medium" or "high" ratios.

iii) Na/(Ca+K) Ratios for Other Test Results

The consultant calculated Na/(Ca+K) ratios for all toxicity test data in which chemical analyses were available. It was reported that the ratios were not normally distributed and therefore decided that statistical analysis of the ratios would be postponed.

Graphical comparisons of trout and daphnid mortality to the calculated Na/(Ca+K) ratio suggested that a Na/(Ca+K) threshold may exist for *Daphnia magna* at a ratio of approximately 75:1. Eighty to one hundred per cent daphnid mortality occurred at Na/(Ca+K) ratios greater than 75:1. At Na/(Ca+K) ratios less than 28:1, mortality ranged from 0 to 40%. Two outliers were observed; 97% at a ratio of 40:1 and 80% mortality at a ratio of 66:1. A Na/(Ca+K) threshold did not appear to exist for rainbow trout. However, the consultant noted that an insufficient number of toxic samples were obtained during the study period to confirm or reject the hypothesis.

Na/Ca and Na/K ratios were also calculated. Eighty to one hundred per cent daphnid mortality occurred at Na/Ca ratios greater than 60:1. At Na/Ca ratios less than 40:1, mortality ranged from 0 to 40%. One outlier was observed; 67% mortality at a ratio of 113:1. Eighty to one hundred per cent daphnid mortality occurred at Na/K ratios greater than 624:1. At Na/K ratios less than 195:1, mortality ranged from 0 to 40%. As with the Na/(Ca+K) ratios, a threshold did not appear to exist for rainbow trout due to an insufficient number of toxic samples tested.

Chemical Equilibrium Modelling

Chemical equilibrium modelling indicated that the addition of calcium to effluent samples, either by passage through a zeolite column or by deliberate addition, led to the formation of calcite ($CaCO_3$), which precipitated. The formation of the precipitate was expected to decrease the concentration of carbonatess. For example, the concentrations of soluble carbonates in the untreated effluent samples collected on March 23 and April 19 were 4.09 and 4.68 mM, respectively. After zeolite treatment, carbonates were predicted to decrease to 2.5 and 3.7 mM. This decrease in carbonates was accompanied by a decrease in effluent toxicity. The samples which were passed through zeolite columns were less toxic than untreated samples. The addition of calcium and potassium, which was done in order to decrease the Na/(Ca+K) ratio, also resulted in a less toxic effluent.

The consultants noted that few studies have associated carbonates with toxicity to aquatic organisms. However, the results of regression modelling supported the inclusion of saturated carbonates as a source of toxicity to *Daphnia magna*. The initial regression model included a term for alkalinity and the final model included the bicarbonate concentration. It was also noted that the mechanism of toxicity due to elevated carbonates was not known. The role of carbonates in trout toxicity was unclear, since an insufficient number of toxic samples was obtained during the study.

Statistical Analysis

Regression modelling was conducted separately for *Daphnia magna and* rainbow trout. The consultant noted that emphasis was placed on analysis of the *Daphnia magna* toxicity data due to the larger number of toxic samples and, consequently, an expected greater chance of producing a significant model. It was also indicated that the methods used in the data analysis were reviewed with a statistician.

Daphnia magna

The preliminary regression model, with a regression coefficient (r^2) of 0.96, included the factors potassium, copper and alkalinity. The equation for the regression indicated that the presence of potassium decreased the toxicity while elevated levels of alkalinity and copper were associated with toxicity. Calcium and magnesium appeared to have little impact on the overall fit of the model.

The preliminary model still included a general chemical parameter "alkalinity". The subsequent regression models replaced the alkalinity term with the components that contribute to alkalinity: initial or final pH, carbonate, bicarbonate, sulfate, silicate and boron components. In addition, the concentrations of bicarbonate and carbonate forms of alkalinity were calculated at the initial and final pH values (e.g., pH measured at the beginning and end of the bioassays). The final model included potassium, copper, bicarbonate with an r^2 value of 0.96. Inclusion of carbonates in the model did not improve the overall r^2 value and in fact was insignificant in the analysis of variance (ANOVA) for the regression. The model assumptions were checked and verified.

Rainbow Trout

Regression modelling with rainbow trout was limited due to the few number of samples which resulted in toxicity. The highest r^2 value (0.6) was obtained for copper. Calcium, magnesium, potassium and alkalinity had little effect on the model. These model assumptions were also checked and verified.

Conclusions of 1998 Study

- Results from the 1998 TIE study were inconclusive. The standard U.S. EPA approach to toxicant identification was not possible since all of the Phase I TIE treatments were ineffective at reducing or eliminating effluent toxicity.

- Treatment with zeolite (typically used during Phase II TIEs) was the only effluent manipulation which consistently reduced or eliminated rainbow trout and *Daphnia magna* mortality. The beneficial effect of zeolite on the effluent was due to the removal of a substance other than ammonia since concentrations were below (<0.05 mg/L) toxic levels.

- The addition of calcium and potassium to the effluent was beneficial to daphnid and trout survival, but was less effective than the zeolite treatments in it's ability to reduce/eliminate toxicity.

- The results suggested that the cause of toxicity was related to a change in the relative concentration of individual components of TDS, rather than elevated TDS alone.

- It was hypothesized that sodium levels were sufficient to account for at least 50% of the *Daphnia magna* mortality. However, the concentration of sodium did not vary enough to be identified as a significant parameter in the regression model.

- Additional potential sources of toxicity, though not obvious from the data, were identified through regression modelling. Copper, carbonates and potassium (ion balance) were considered important factors in explaining daphnid mortality. However, the relative contribution of each factor in explaining toxicity varied between samples, making data interpretation difficult. The model could not be confirmed since only a few of the samples tested resulted in partial daphnid mortalities.

- The existence of a Na/(Ca+K) threshold was supported by the regression modelling which identified potassium as a significant parameter in reducing daphnid mortality. Comparisons of daphnid mortality to the calculated Na/(Ca+K) ratio suggested that a threshold may exist for *Daphnia magna* at a ratio of approximately 75:1.

- Regression modelling with rainbow trout was limited due to the few number of samples which resulted in acute lethality. Based on the limited available data, it was suspected that periodic peaks in sodium (e.g., > 6000 mg/L) and/or copper (e.g., > 0.1 mg/L) concentrations contributed to the sporadic toxicity.

- The test data generated did not support a firm conclusion as to the cause(s) of rainbow trout and *Daphnia magna* mortality. The standard approach to toxicant identification proved ineffective and subsequent toxicant identification efforts will require the continued development of innovative methodologies and techniques.

3.3.5.4 Comments on the 1998 TI/RE Approach

The study appeared to be efficiently managed and thorough in reporting, data analysis and interpretation. However, the specific substances responsible for effluent toxicity were not conclusively identified. Several possible causes of toxicity were reported, however the investigation is not complete and a future course of action is being developed. It was also clear from the report that the cause of effluent toxicity was atypical for an effluent associated with mining in Canada (e.g., toxicity was not related to pH or ammonia) and that the "standard" U.S. EPA Phase I treatments were not effective.

i) Approach to Phase I TIEs

A complete comprehensive report was prepared for the client. The report included all toxicity and chemical data. Descriptions of the treatment methods and test conditions (e.g., exposure volumes, number of organisms per replicate) were also provided and aided in the interpretation of the test data.

The consultant followed the standard Phase I TIE approach, but also incorporated the use of zeolite, activated carbon, XAD, cation and anion exchange resins. Due to the inability to treat large volumes of effluent, the C18 columns, XAD resin, cation and anion exchange resins were only used during Phase I TIE testing with *Daphnia magna*. The resin treatments had no effect at reducing effluent toxicity and the post treated effluent samples were not submitted for chemical analysis. The chemical data from these treatments may have provided more information on the relative contribution of the various ions (e.g., Ca, Na, K, Mg, carbonates) to toxicity of the effluent. However, the environmental coordinator for the mine indicated that cost considerations were a factor in determining the number of samples submitted for chemical analysis.

The standard U.S. EPA Phase I TIE includes filtration at pH i, 3 and 11 using a 1.0 μm glass fibre filter. It was noted during this review that the filtration at pH 11 reduced toxicity of the effluent to rainbow trout (100% mortality in the untreated effluent and 60% in the pH 11 filtered effluent). However, the post treated effluent was not submitted for chemical analysis. It is unclear from the report why the samples were not analysed. An effluent sample was also filtered at pH i (pH 9.2) using a 0.45 μm filter (glass fibre with binder) to provide information on the type of filterable material that may be responsible for effluent toxicity. In general, any substance that is smaller than 0.45 μm is considered to be dissolved and "biologically" available. Larger particles may be less biologically available, but can cause toxicity through ingestion. During this study, filtration of the effluent using a 0.45 μm filter did not reduce trout or daphnid mortality. However, this treatment may have been more effective if the sample had been filtered at a higher pH (e.g., pH 11) where a larger proportion of the contaminants would be present in particulate form.

The consultant noted in the report that the causes of trout and daphnid toxicity were considered separately. The rationale was based on the fact that not all samples were toxic to both species and *Daphnia magna* were generally more sensitive than rainbow trout. While this approach was logical, repeated testing with *Daphnia magna* on the sample which was toxic to trout may have clarified if the organisms were responding to different toxicants, or if they differ in their sensitivity to the same toxicant(s). Specifically, the first sample collected for Phase I testing was lethal to *Daphnia magna*, but nonlethal to rainbow trout (Phase I testing was conducted using daphnids only). The second sample was lethal to trout, however the sample was not assessed for it's toxicity to *Daphnia magna* (Phase I testing was conducted using trout only).

ii) Chemical Analysis

The analytical method detection limits (MDLs) were sufficiently low so as not to present an impedance to data interpretation and the detection of pertinent subtle changes in chemistry. However, it was noted by the consultant that MDLs varied between samples due to interferences present in the effluent. Most samples required dilution in order for all parameters for a given batch to be successfully analysed. For a given set of parameters (e.g., anions or metals), the analysis was conducted using an automated system in which all measurements (peaks) were required to fall within the same range. If this did not occur, the sample was diluted until all parameters could be measured on the same scale. It was noted that samples with elevated chloride (in the case of the anion analysis using ion chromatography) and elevated TDS (in the case of the ICP/MS (inductively coupled plasma/mass spectrometry)) resulted in interferences with the automated analysis of samples. However, it did not appear that differences limited the data analysis, since the main parameters of interest did not approach the MDLs. It may have been useful to assess the most cost effective and sensitive analytical method prior to initiation of the TIE study. For example, although ICP has higher detection limits, it is not as sensitive as ICP/MS to samples with high TDS and therefore may have provided similar MDLs.

iii) Ion balance experiments

The ion balance experiments (proposed and designed by a researcher at a local university) included the addition of both calcium and potassium to the effluent samples. However, testing should have included the addition of Ca or K individually. This information would have clarified which of the two cations had a greater effect at reducing toxicity. The consultants did note that additional informal experiments were conducted using calcium (as calcium sulfate) alone. The toxicity data indicated that these additions also reduced toxicity, however samples of the treated effluent (Ca additions alone) were not submitted for chemical analysis.

iii) LC50s versus single concentration tests

All of the tests involved single concentration exposures to the full strength (100%) effluent. The historical data suggested that this approach was acceptable for tests involving rainbow trout, since toxicity was not typically observed in the diluted effluent (e.g., 50, 25% concentrations). LC50 tests may have been beneficial for tests conducted with *Daphnia magna* since the historical data indicated that toxicity was often observed in the diluted effluent concentrations. The LC50 test has an advantage over single concentration tests in that subtle reductions in effluent toxicity can be detected. This information can be important when a treatment does not eliminate toxicity, but instead reduces the effect of a particular toxicant. However, conversations with mine personnel indicated that differences between effluent and dilution water quality was the main rationale for avoiding LC50 testing (e.g., the effluent was harder and contained more dissolved solids compared to a typical laboratory dilution water). This rationale was supported by the U.S. EPA guidance document which provides a cautionary note regarding the potential confounding effects of dilution water on effluent toxicity. However, little guidance regarding the selection criteria for dilution water was provided by the U.S. EPA. In place of LC50 testing, the zeolite treatment was replicated in order to provide sufficient data for statistical analysis as well as information on effluent variability.

3.3.5.5 Overall Summary of Case Study

- The environmental coordinators were satisfied with the results from all studies, but indicated that limitations with the TI/RE process existed making toxicant identification complex. This study was considered to be well planned and involved a multi-disciplinary team (government scientists, consultants, researchers, mine personnel), however the standard Phase I approach proved ineffective and the specific substance(s) responsible for toxicity were not identified.
- Studies to investigate the cause of effluent toxicity were conducted between 1996 and 1998. The refinery complied with all regulatory effluent chemical limits, but still experienced periodic failures with rainbow trout and *Daphnia magna* acute toxicity testing.
- Treatment with zeolite was the only effluent manipulation which consistently reduced or eliminated rainbow trout and *Daphnia magna* mortality. The addition of calcium and potassium to the effluent reduced daphnid mortality, but was less effective compared to the zeolite treatments.

- Chemical analysis of the untreated and treated effluent samples indicated that zeolite increased the effluent concentration of calcium, potassium, magnesium and strontium and slightly reduced the effluent pH as well as the concentration of various trace metals (e.g., copper, cobalt), carbonates and bicarbonates.
- The TDS concentration in the zeolite treated effluent remained similar to the untreated effluent suggesting the cause of toxicity was related to a change in the relative concentration of individual components of TDS, rather than elevated TDS alone.
- It was hypothesized that sodium levels were sufficient to account for at least 50% of the *Daphnia magna* mortality. Copper, potassium and carbonates were identified as potentially important factors in explaining daphnid mortality. Atypical ion balance was also a suspected cause of daphnid toxicity.
- Based on the limited available data, it was suspected that periodic peaks in sodium (e.g., > 6000 mg/L) and/or copper (e.g., > 0.1 mg/L) concentrations contributed to the sporadic toxicity.
- The standard approach to toxicant identification was not possible since the U.S. EPA Phase I TIE treatments were ineffective at reducing or eliminating effluent toxicity. Subsequent toxicant identification efforts are in progress, but have required the development of innovative methodologies and techniques.

Table 3-19. Summary of chemical analysis on untreated and treated samples collected for *Daphnia magna* Phase I TIE. Measured parameters not provided were reported as less than the MDL.

Sample (Date Tested)	untreated effluent		Zeolite rep. #1		Zeolite rep. #2		Carbon	
	unfiltered	filtered	unfiltered	filtered	Unfiltered	filtered	unfiltered	filtered
pH (initial)	9.1		8.9		8.9		9	
pH (final)	8.9		8.8		8.7		9	
TOC	4.4		4		4.5		1.1	
DOC	4.2		3.9		4.4		1.2	
TDS	7880		7920		7680		7900	
Alkalinity (as $CaCO_3$)	350		316		290		390	
Bicarbonate (as HCO_3)	325		298		276		356	
Carbonate (as CO_3)	24		17.7		13		33	
Chloride	974	1000	973	1000	985	1000	969	963
Sulphate (as SO_4)	4020	4060	4020	4040	4020	4020	3960	3910
Al	0.012	0.01	0.012	0.011	0.018	0.015	0.157	0.153
As	0.132	0.127	0.131	0.131	0.129	0.125	0.14	0.139
B	0.834	0.796	0.826	0.818	0.79	0.753	0.363	0.0352
Ca	19.5	19.2	77	77.4	76.1	89.1	20.6	20.1
Cr	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05
Co	0.0581	0.0473	0.0281	0.0228	0.0259	0.0284	0.0146	0.0126
Cu	0.047	0.041	0.022	0.018	0.024	0.028	<0.005	<0.005
Fe	<0.03	<0.03	<0.03	<0.03	<0.03	<0.03	<0.03	<0.03
K	4.1	4.1	25.8	26.2	26.1	26.9	4.3	4.2
Mg	15.9	16.2	26.1	26.2	25.6	26.3	15.8	15.6
Mn	0.062	0.047	0.022	0.014	0.02	0.03	0.019	0.015
Na	2470	2410	2230	2340	2260	2340	2460	2400
Ni	0.192	0.158	0.142	0.125	0.136	0.126	0.051	0.044
Pb	0.0011	0.0008	0.0005	0.0005	0.0005	0.0006	0.0006	0.0005
S	1370	1380	1380	1330	1360	1370	1340	1320
Sb	0.0025	0.0024	0.0025	0.0025	0.0025	0.0019	0.0034	0.0033
Se	0.074	0.071	0.074	0.073	0.074	0.072	0.071	0.072
Si	6.75	6.66	7.19	7.25	7.15	7.25	6.88	6.65
Sr	0.139	0.135	1.47	1.47	1.48	1.48	0.224	0.226
V	0.014	0.014	0.013	0.013	0.013	0.013	0.022	0.023
Zn	<0.002	0.005	<0.002	<0.002	<0.002	<0.0020	0.002	0.004
Hardness (as mg $CaCO_3$ /L	114	115	300	301	295	331	117	114
% mortality (% immobile)	100		40		13 (3)		90	

Note: carbonate and bicarbonate were calculated using pH values at test completion

Table 3-20. Summary of chemical analysis on untreated and treated samples for rainbow trout Phase I TIE. Measured parameters not provided were reported as less than the MDL.

Sample (Date Tested)	untreated effluent		Zeolite rep. #1		Zeolite rep. #2		Carbon	
	unfiltered	filtered	unfiltered	filtered	unfiltered	filtered	unfiltered	filtered
pH (initial)	9.2		9.1		9.1		9.1	
pH (final)	9.1		9		9.0		9.0	
TOC	3.5		3.9		3.8		0.9	
DOC	3.3		3		3.2		<0.5	
TDS	9110		8760		9020		9130	
Alkalinity (as $CaCO_3$)	456		364		358		468	
Bicarbonate (as HCO_3)	407		332		327		427	
Carbonate (as CO_3)	48		31		31		40	
Chloride	819	811	794	830	801	788	839	804
Sulphate (as SO_4)	4620	4720	4600	4700	4670	4480	4670	4560
Al	<0.05	<0.05	<0.05	<0.05	0.07	<0.05	0.18	0.18
As	0.12	0.12	0.12	0.12	0.12	0.11	0.13	0.13
B	0.64	0.65	0.62	0.61	0.59	0.61	0.11	0.1
Ca	13	13	70	70	79	74	12	12
Cr	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05
Co	0.113	0.066	0.056	0.029	0.049	0.035	0.026	0.011
Cu	0.198	0.107	0.109	0.059	0.097	0.057	0.022	<0.005
Fe	0.3	--	0.4	0.4	0.3	0.3	0.4	0.3
K	5	6	19	19	19	19	4	5
Mg	6.8	7.5	18	17.8	21	20.6	7.3	7.6
Mn	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05
Na	3090	3440	3020	3000	2940	2880	3010	3110
Ni	0.22	0.12	0.15	0.08	0.12	0.08	0.05	0.02
S	1610	1680	1580	1580	1600	1670	1580	1580
Sb	<0.005	0.01	0.006	0.006	0.005	<0.005	<0.005	<0.005
Se	0.11	0.12	0.11	0.11	0.11	0.11	0.11	0.11
Si	6.8	7.5	6.8	6.7	6.7	6.5	6.9	7
Sr	0.08	0.08	1.12	1.12	1.22	1.2	0.13	0.13
V	0.008	0.01	0.008	0.008	0.008	0.008	0.014	0.014
Zn	<0.02	<0.02	<0.02	<0.02	0.08	<0.02	<0.02	<0.02
Hardness (as mg $CaCO_3$/L)	60	63	249	248	284	270	60	61
% mortality	100		0		0		0	

Note: carbonate and bicarbonate were calculated using pH values at test completion

Table 3-21 **Results from calcium chloride and potassium chloride additions - testing using *Daphnia magna*.**

Treatment	% mortality	% immobile	Approximate Na/(Ca+K) ratio based on measured values
untreated sample	100	0	96:1
Ca, K additions ("low" ratio)	37	3	15:1
Ca, K additions ("medium" ratio)	97	0	40:1
Ca, K additions ("high" ratio)	93	3	88:1

Table 3-22. **Results from calcium chloride and potassium chloride additions - testing using rainbow trout.**

Treatment	% mortality	LT50 (hrs)	Approximate Na/(Ca+K) ratio based on measured values
untreated sample	90	24	219:1
Ca, K additions ("low" ratio)	10	>96	15:1
Ca, K additions ("medium" ratio)	0	>96	60:1
Ca, K additions ("high" ratio)	30	>96	87:1

3.4 Summary and Description of Successful TRE Treatment Options

Two successful effluent treatment options were encountered during the case studies and survey questionnaire including; pH adjustment (control) and chemical substitution. These treatments were reported as successfully eliminating or reducing effluent toxicity. One mine (copper/nickel) and one refinery reported that the effluent had been re-routed to an alternate treatment facility with a longer retention time. Although this approach was not considered a direct effluent treatment option, a longer retention time may help reduce toxicity by allowing for the natural degradation of ammonia and settling of solids.

Based on the large number of mines who reported (in the survey) nonlethal discharges, it could also be concluded that there are likely other successful effluent treatment approaches that can result in the reduction or elimination of effluent toxicity. However, this assumption (with respect to the survey results) must be interpreted with caution since it is unknown how often each mine assessed effluent toxicity. It should also be noted that approaches to effluent treatment will be site and contaminant specific and describing all possible treatment options was beyond the scope and budget of the current study

pH adjustment

pH control was used for several reasons; to increase or reduce pH such that the effluent was within the regulated discharge limits, to ensure that pH alone was not the cause of toxicity, to clarify the effluent and to reduce un-ionized ammonia concentrations below lethal thresholds. Adjustment of pH was most commonly achieved by the use of lime ($Ca(OH)_2$) (e.g., for metals/solids removal) or with carbon dioxide or sulphuric acid (e.g., to meet chemical/toxicological discharge limits).

In case study #1, the treatment system includes the addition of lime to reduce suspended solids. The volume and capacity of the clarifier/settling pond was expanded and pH control (e.g., addition of lime) was improved. In case study #3, compliance with effluent discharge limits for pH were achieved by adjustment of the effluent using sulphuric acid produced at the mine site. Effluent pH was also critical to the reduction of effluent toxicity caused by un-ionized ammonia. However, it was noted that reduction of pH increased effluent toxicity to daphnids and was likely due to the increased bioavailability of metals at lower pH. A gold mine reported in the survey that carbon dioxide was used to lower effluent pH in order to reduce the concentration of un-ionized ammonia. Carbon dioxide in liquid form was vaporized before being introduced into the wastewater. The Ontario Ministry of the Environment (1993) reports that carbon dioxide has the advantage of reduced health and safety risks, is comparable to mineral acids in terms of cost and has been demonstrated to minimize overshoots.

Chemical substitution

In case study # 4, the mine effluent consists of three process streams: "LIX", "raffinate" and "mine water". The mine operation involves an extraction and recovery step for solvents. In fact, when the effluent became toxic, mine personnel suspected that loss of solvent to the effluent was the cause of toxicity. The TIE study successfully identified the compound responsible for toxicity, a compound which was present in the two of the process streams (isodecanol). The mine conducted an in-house TRE following the TIE study in order to reduce isodecanol use and substitute with other types of solvents. The steps also included increased treatment efficiency (e.g., use of increased aeration) and toxicity monitoring using the Microtox® system.

3.5 Novel TIE Manipulations

The main objective of this task was to provide a summary and description of novel effluent treatment techniques encountered during the literature review, survey and case studies. However, techniques other than the standard TIE treatments were not encountered.

Based on our own TI/RE experience, we have found exchange resins useful in toxicant identification. The anion and cation exchange resins are typically used to remove ionic species from aqueous solutions. However, these resins may also remove organic ions of high molecular weight and metallic anionic complexes. If observed to remove toxicity, then the columns can be eluted (rinsed) using deionized water, sodium hydroxide or hydrochloric acid. XAD resin can remove a broad range of organic contaminants. The resin is supplied as insoluble white beads and will remove organic substances of relatively low molecular weight. If observed to remove or reduce toxicity then the column can be eluted using different solutions including; deionized water where adsorption is from an ionic solution; methanol for hydrophobic organics, dilute sodium hydroxide for weakly acidic substances or dilute hydrochloric acid for weakly basic substances. Properly treated controls are also important when using resins, since the columns can release organic toxicants.

3.6 Benefits and Limitations of Conducting TI/REs

Many of the mines which reported having conducted a TI/RE provided general comments regarding their experience as well as a list of the perceived strengths and weaknesses of the TI/RE process (Appendix D). In fact, the strengths and weakness reported by mine personnel (summarized in Table 3-23) represented many of the benefits and limitations of the TI/RE process. However, one of the most important benefits not reported in the survey questionnaire is that the TI/RE process incorporates the responses of organisms into the assessment of complex effluent mixtures to determine the identity of the substance(s) responsible for toxicity. Attempts to use chemical screening alone to identify substances responsible for effluent toxicity are typically unsuccessful for several reasons. First, it is difficult and costly to ensure that all possible contaminants will be identified and measured. Secondly, even if the chemicals of concern are measured, toxicity data may be unavailable for the majority of detected substances. Lastly, it is difficult to predict the additive, antagonistic and/or synergistic interaction that may occur among the contaminants. The TI/RE process allows matrix effects and toxicant bioavailability to be quantified (Ankley *et al.*, 1992; USEPA 1991a). The process also allows for increased analytical precision and sensitivity by providing the characteristics of the suspected toxicants. Without some knowledge of the toxicant, broad spectrum analyses (e.g., GC/MS, HPLC) are less sensitive and costly.

As discussed previously, the "standard" TI/RE approach can be difficult to apply when effluent toxicity is not persistent or transient. In these instance, alternative approaches are required to determine the cause of toxicity (e.g., on-site testing, rapid micro-scale tests, direct treatment approach). Identification of the cause of toxicity can be complex when more than one substance is suspected, or when matrix effects are involved. If these identification and confirmation studies are to be successful, it is crucial that the tests are well planned and scientifically defendable. It is at these stages of the TI/RE study that the experience of the investigator is crucial.

It must also be recognized that conclusions as to the cause of toxicity cannot be based on a single sample. If the suspected cause(s) of toxicity is identified, repeat testing on different effluent samples must be conducted in order to account for effluent variability and confirm that the cause of toxicity is the same under all conditions.

Table 3-23. Summary of TI/RE benefits (strengths) and limitations (weaknesses)

Benefits (Strengths)

- TI/RE process enables evaluation of toxicity components in effluent;
- focuses attention on the particular chemical groups responsible for toxicity;
- a systematic approach to solving toxicity problems;
- useful process when used in conjunction with the knowledge of the process (e.g., changes, upsets, reagent usage etc.);
- identification of toxicant characteristics can lead to treatment changes and a reduction/elimination of toxicity (e.g., toxicity treatability approach); and
- on-site testing/monitoring is possible in cases of non persistent or transient toxicity, if a correlation can be established between the rapid-screening test and the species of interest.

Limitations (Weaknesses)

- process can be more difficult if toxicity is transient;
- process was not designed for cases where effluent toxicity is not persistent;
- results can be difficult to obtain when more than one toxicant is involved;
- in complex effluents, synergism effects are complicated and can be difficult to identify;
- more research is required to identify and develop TI/RE methods specific for mining effluent contaminants;
- results are variable for chronic toxicity where levels of contaminants are low;
- require more guidance on what to do with results of Phase I TIE manipulations - knowledge and experience of investigator is critical if you are to get beyond “speculating” stage; and
- the lack of understanding by the two groups, TIE specialists and mining group, of each other’s processes can lead to the incomplete transfer of information (only a limitation if groups do not communicate).

4. CONCLUSIONS AND RECOMMENDATIONS

Published literature on TI/REs was not extensive and there were very few studies which dealt with TIEs conducted for the mining industry. It was concluded that the overwhelming majority of TIE studies are never published in the scientific literature for various reasons and is unfortunate, since this limits the exchange of information which is vital if the field is to advance.

A complete assessment of the Canadian mining sector s experience with the TI/RE process was not possible since less than 50% of mines responded to the survey. Ammonia and metals were the most commonly identified causes of effluent toxicity. Toxicity was also often dependent on pH (e.g., toxicity due to ammonia at high pH and metals at low pH). Most mines reported that primary toxicants were easily identified, but secondary causes of toxicity were often based on speculation. Dissatisfaction with the TIE process appeared to be partially related to lack of identification and confirmation of these secondary toxicants. A high degree of certainty as to the cause of effluent toxicity is required before potentially costly changes at the plant scale can be implemented. Yet many mines reported not going beyond the Phase I TIE.

The U.S. EPA Phase I TIE was designed as a generic approach to be used with any industrial effluent. However, modifications to the standard U.S. EPA Phase I TIE approach were noted in several of the case studies. For example, many of the treatments appeared to focus on elevated pH, ammonia and metals, the most common causes of mining effluent toxicity identified in this study. Based on the results from the survey and case studies it would be useful to develop TIE approaches specific for the Canadian mining industry. First, it would be worthwhile to modify the Phase I treatment approach such that the most "common" toxicants associated with mining effluents are tested for at the start of the Phase I study. Other treatments could be excluded until such time that the "common" toxicants are ruled out as the primary causes of toxicity. Treatments with anion and cation exchange resins should be refined and included in the modified battery of Phase I treatments.

Secondly, the expense associated with shipping large volumes of effluent for trout testing and the use of fathead minnows as a surrogate test organism could be avoided if the Environment Canada acute lethality test with trout was modified for use in TIEs. For example, several studies reported the use of reduced exposure volumes with fewer fish per concentration, however the approach was varied and often not well documented. Further investigation is required to ensure that modifications to the Environment Canada protocol will generate valid and reproducible results. As mentioned previously, a modified TIE approach using rainbow trout has been investigated by the pulp and paper industry and the results may provide useful guidance for mining TIEs.

Responses to the survey and case studies clearly indicated that full transfer of information and communication between the mine and testing laboratory is critical to the success of a TI/RE study. The identification of the source or cause of effluent toxicity will increase greatly when mine personnel actively participate in the TI/RE study. Based on results from the case studies and our own experience, we have provided a basic list of questions to assist mine personnel to increase the success of their TI/RE investigation. The positive and negative TI/RE approaches used in the case studies and responses to the survey questionnaire were taken into consideration when compiling the list of questions. It is hoped this type of information can provide focus to the TI/RE investigation and help identify the substance(s) responsible for toxicity.

1. **Review of toxicity and chemistry data:**

- is toxicity persistent?
- is toxicity transient?
- is transient toxicity related to mine processes, time of year?
- are there differences in species sensitivity?
- are there any "obvious" toxicants (e.g., un-ionized ammonia, metals, pH)?
- is there any correlation between toxicity and specific water quality parameters (e.g, D.O., pH, conductivity) measured in the toxicity bioassay?
- have changes to test protocols (e.g., changes to aeration rates) been taken into consideration when reviewing toxicity data?
- have split samples been submitted for toxicity testing at different laboratories and are the results the same or different? If the results are different can they be explained (e.g., differences in dilution water characteristics)?
- are the characteristics of the effluent stable (e.g., is the effluent at equilibrium)?
- is the effluent sufficiently toxic to conduct a TIE study (e.g. at least complete mortality in the 100% effluent)?

2. **Review of plant operation/effluent treatment system:**

- has the operation changed recently?
- did any known spills occur during the toxic event?
- is the effluent treatment system operating properly?
- have any new treatment chemicals been used during the toxic events?
- have different inputs to the treatment plant been assessed for toxicity and analyzed chemically?

3. **TIEs:**

- if a surrogate species is used, are there sufficient data available indicating a similar sensitivity to the species of interest?
- is the rationale for modifications to the Phase I TIE treatment logical?
- are sufficient chemical data to be gathered and then compared to toxicological results?
- are both total and dissolved parameters included in chemical analyses?

- does the toxicant appear pH dependent and, if so, will increased pH monitoring be included during the toxicity tests?
- is toxicity modified by water quality parameters (e.g., pH, hardness, alkalinity) as these may confound interpretation of LC50 results?
- are samples for chemical analyses representative of the effluent to which the organisms were exposed?
- are identification and confirmation studies designed to test specific hypothesis (e.g., what answers will the studies provide)?
- have a sufficient number of samples been tested (and replicated) to account for effluent variability and confirm the cause(s) of toxicity?
- have appropriate statistical analyses been conducted?
- have the raw test data been reviewed by mine personnel?

4. **Effluent treatability:**

- is toxicity testing included in all bench scale and pilot plant studies (e.g., to ensure treatment is effective or that treatment for one toxicant does not facilitate the expression of another toxicant)?
- can toxic streams (e.g., mine water) be isolated?
- are mine personnel, toxicologists, chemists and engineers involved in the investigation aware of what each group has found?
- were effluent samples chemically analyzed on-site immediately after treatment and at the start of toxicity testing?
- were pilot plant trials tested under a variety of effluent conditions?

5. GLOSSARY

acute:	happening within a short period of time, usually taken as ≤ 96 hours for fish or ≤48 hours for daphnids
chronic:	occurring during a relatively long period of exposure, usually a significant portion of the life span of the organism
LC50:	concentration of material in water that is estimated to be lethal to 50% of test organisms after a defined period of exposure
lethal:	causing death by direct action
pH_i:	the initial pH of the effluent as received, before adjustment
sublethal :	detrimental to an organism, but below the level which directly causes death within the test period
toxicity :	the inherent potential or capacity of a material to cause adverse effects in living organisms
toxicity identification evaluation:	set of physical and chemical manipulations used to identify the specific chemical (or it's characteristics) responsible for the toxicity of effluents
toxicity reduction evaluation:	site specific study conducted to identify the causative agents in a toxic effluent, isolate the sources, evaluate the effectiveness of control options, and then confirm the reduction in effluent toxicity

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APPENDIX A

EVALUATION CRITERIA AND CASE STUDY CHECKLIST

EVALUATION CRITERIA

Are the objectives or targets of the TRE clearly and accurately stated?

Are the approaches or methods to be utilized described to the extent that is possible prior to beginning the TRE?

Has available EPA guidance been utilized in the design of the TRE and development of the TRE plan (or if other methods are proposed, are these sufficiently documented)?

Does the TRE plan specify what results and data are to be included in the interim and final reports?

Are the toxicity test methods and endpoints which will be used described or referenced?

Does the approach described build on previous results and proceed by narrowing down the possibilities in a logical progression?

Does the plan provide for all test results to be analyzed and used to focus on the most effective approach for any subsequent source investigations, treatability studies, and control method evaluation?

Are optimization of existing plant/treatment operations and spill control programs part of the initial steps of the TRE?

Does the TRE plan allow a sufficient amount of time and appropriate level of effort for each component of the study plan?

Does the TIE use broad characterization steps and consider effluent variability?

Is toxicity tracked with aquatic organism toxicity tests throughout the analyses?

Is the choice of toxicity tests for the TRE logical and will correlations be conducted if the species used are different from those used for routine biomonitoring?

Is the laboratory analytical capability and the expertise of the investigator broad enough to conduct the various components of the evaluation?

Was the TIE/TRE work performed by qualified personnel?

Was an accredited toxicological and/or analytical laboratory utilized?

Were standardized test procedures utilized?

If novel approaches were taken, was the methodology designed such that the data would be reasonably reliable?

What kind of end-of-pipe effluent treatment was in place prior to the TRE?

Were the mine or effluent treatment operators interviewed or questioned prior to TRE/TIE initiation as to the performance of the treatment system or recent changes in mine operations or treatments?

Was a site inspection conducted to evaluate internal Ahouse-keeping@ practices (i.e. chemical disposal etc.)?

Were treatment or process chemicals examined prior to initiation of the TIE?

Was toxicity of intake water assessed?

Were all sources of discharge to treatment system identified (i.e., process effluent, cooling water)?

How often were the Phase I treatments repeated and were the results the same each time?

How was pH maintained during testing?

Statistical validity? Were statistics used in Phase I, Phase II or Phase III? Should they have been used?

TI/RE CHECKLIST USED TO EVALUATE EACH CASE STUDY

Type of Mine	Effl. Treat.	TIE / TRE	Phase (1,2,3)	Spp [a] Used	Treatments [b]										Cost (000)	Success [c]
					pH adjust	pH + Filt=n	pH + Air	pH, Grad.	pH + C18	Ion Exch.	Act. C/ XAD-4	Zeolite	EDTA Chel=n	Na Thios.		

[a] Test species:

Dm: *Daphnia magna*
Cd: *Ceriodaphnia dubia*
Rt: rainbow trout
Fm: fathead minnow

[b] TIE Treatments:

- pH Adjustment
- pH Adjustment/Filtration
- pH Adjustment/Aeration
- pH Adjustment / Graduated pH
- pH Adjustment / C18 Adsorption
- Cation / Anion Exchange
- Activated Carbon / XAD-4
- Zeolite
- EDTA Chelation
- Sodium Thiosulfate Treatment

[c] TIE success = toxicant identified; TRE success = toxicant eliminated/removed from effluent

APPENDIX D

COMMENTS FROM SURVEY

General comments regarding experience with the TIE/TRE process as reported by mine personnel

The TIE was a positive experience because it produced conclusive results following the Phase I.

When we did our own testing of consequences of pH adjustment we found that lower pH reduced toxicity.

Primary candidates for toxicity can be easily identified, but secondary toxicants make it difficult to develop appropriate action;

AScience@ of effluent testing is not developed enough for toxicants that aren=t obvious.

In complex effluents, synergism effects are complicated and unknown.

Effluent chemistry and toxicity was not correlated and conclusions were based on speculation and literature from studies of non-complex mixtures;

The TIE program showed sufficient correlation between acute toxicity and Microtox, that an internal control mechanism based on Microtox was implemented. Microtox results have resulted in re-treatment of effluent to control effluent and receiving water toxicity;

Toxicity not persistent - effluent became nonlethal immediately after initial toxicity tests and we therefore abandoned sending multitudes of buckets to toxicity lab every month.

It was felt that better return on investment was obtained when LC50's were not used and instead used more single concentration tests with effective manipulation from the TIE work. We concentrated more on chemistry/toxicity correlations where very obvious reductions in toxicity were observed. TIE=s are very difficult to coordinate when toxicity and/or effluent chemistry is inconsistent - results are only good for the chemical mix encountered the day the sample was taken. Effluent chemistry can change dramatically in a relatively short time period. Chemical and toxicological results may not be synchronized, throwing off interpretation and relevancy to actual conditions in receiving water. Pre-test aeration can change effluent chemistry.

TIE process does not always provide enough evidence or information to confidently engineer a solution. The toxicity of complex mixtures is not understood well enough - lots of guess-work at times. In-plant changes can put you back to Asquare-one@ if the effluent characteristics change.

Our problem is not with the TIE itself, but with the legislation. When we reduce the pH to 7 with CO_2, we meet the ammonia limit. However, the toxicity test protocol that must be used causes a pH rise with our water. We then fail the toxicity test because of this pH rise. Our water is not toxic if we run a modified US EPA toxicity protocol that uses refresh water instead of aeration.

Mine closed in 1993. TRE/TIE test work has not be completed since 1994. Had budgeted funds to do so in 1996 but the sampling did not occur. A lot of money was spent with little hard evidence to determine what was the cause of lethality. Ammonia and copper were the suspected cause of toxicity, but technology is not available to treat large volumes. Discharge from acid mine drainage (AMD) is still acutely toxic, and seems to be seasonal (eg. more toxic in spring and fall).

Part of our problem was seasonal operation and toxic results occurring during cold weather months. We would have toxic results to *Daphnia magna* in October then effluent would be shutting down for season. Effluent has been non toxic now for two seasons as a result of a different feed source.